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Reactions of dialdehyde starches and wheat proteins

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REACTIONS OF DIALDEHYDE STARCHES AND WHEAT PROTEINS

by

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ABSTRACT

The reaction of dialdehyde starches and the wheat proteins was attempted with twin objectives: 1) to obtain a high polymeric product (plastics, adhesives, rubbers, films, etc.) of commercial importance and 2) to find economic utilization of both wheat gluten and dialdehyde starches.

Vital wheat gluten contains at least nine different kinds of proteins in a highly complicated molecular structure, which is yet to be explored. The other reactant, DAS, which is the periodate oxidation product of the parent starch, is, however known both chemically and structurally.

Because of the handicaps of unknown molecular structure of gluten and non-existence of a very good solvent for both of these reactants, the first phase of the work was somewhat trial and error type -- with the use of different dispersants for gluten and DAS. After a lot of such painstaking trial and error research, the product obtained was a good adhesive for wood to wood bonding.

With the idea about the dispersants for the reactants (e. g. 5% acetic acid for gluten and 5% sodium bisulfite solution for DAS), and the reaction conditions, the experiments were channeled towards optimizing the reaction variables to obtain the best possible adhesive. This was done by carrying out the adhesion experiments using the best reaction product. These experiments were designed by using statistical model and the results were analyzed statistically. These experiments not only gave conclusions to the optimum conditions, but

also gave other valuable information. The factor levels at which interaction of variables would occur, were detected. It was also proved that the glued joints did not show any tendancy to decay in strength with time.

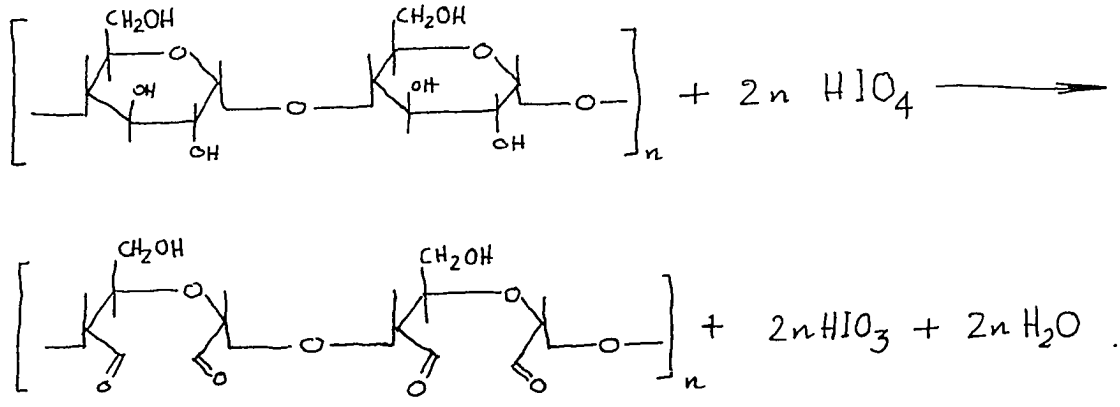
The mechanism involved in the reaction was studied. Regular analytical methods and controlled degradation experiments carried out using the gluten DAS product, were sufficient to locate the most probable aldehyde group in DAS, which was involved in the crosslinking phenomena. Although it was realized that more work on this scheme of mechanism would be optimistic, only if the structure of gluten was known, these investigations yielded quantitative information about the reactivity of the dicarbonyl units in dialdehyde starches.

INTRODUCTION

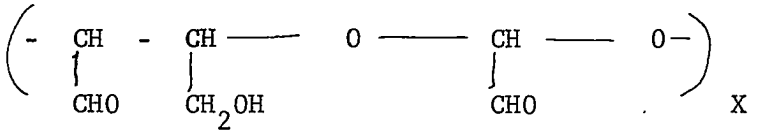
The fact that aldehydes are very good tanning agents (i.e. react with proteins) gave us the first impetus to think that a very high polymeric compound could be made by the reaction of a polymeric aldehyde (e.g. dialdehyde starches) and a high molecular weight protein (e.g the complex wheat protein).

The reaction of gluten and DAS has not been tried by any of the previous workers, who has been researching on the reactions of aldehydes and proteins. Previous work was mainly conducted with an aim to understand the tanning reaction excepting a very few cases, where dialdehyde starch itself was used to effect irreversible insolubilization of casein. On the contrary, the research on the possible reaction between gluten and DAS is an attempt to synthesize a plastic, an adhesive, a rubber or a film and at the same time effect an economic and unique utilization of dialdehyde starches (obtainable from starches by periodic acid oxidation method) and the cheap proteins from wheat.

Dialdehyde starch, a finely divided, practically odorless solid very similar in appearance and color to the original starch, was synthesized in the laboratory by Hudson and Jackson (1).

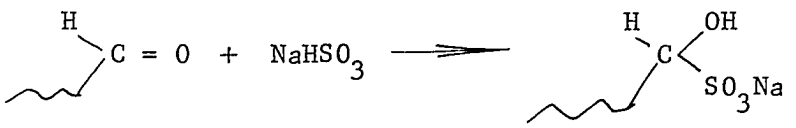


Dialdehyde starches, unlike starch, have extremely interesting chemical properties. These may be attributed to the structure of the substance. It should be noted that for simplicity this structure represents the amylose portion of the original starch (1,4-branching), even though the amylopectin (3-, or 6-branching) is also present. To indicate that DAS is no longer starch, the following formula is used, showing why this product can be referred to as a "polymeric dialdehyde".



These reactive aldehyde groups are the key to the great interest in these compounds. They will react with or crosslink proteins, cellulose, methyl cellulose, starch, dextrins and other polysaccharides, polypeptides and polyhydroxy materials.

Solutions can be made by heating with mild alkali. If a strong alkali is used, the chain can be ruptured to give fragments that will not have the same properties. Solubilization can also be achieved in buffers such as borax or acetate. Mehltretter et al succeeded in preparing the soluble bisulfite addition product (2). Following Mehltretter's method, we have been able to simplify the process by using 5% $NaHSO_3$ solution (in distilled water) as a solvent for DAS. Then the nature of addition of $NaHSO_3$ to any aldehyde group is of the following type:



The Na ion thus inserted is responsible for water solubility of the addition product, even though the original aldehyde is insoluble.

DAS can now be produced in large scale under the patents of Dvonch and Mehltretter (3). This process utilizes an electrolytic regeneration of the expensive periodic acid. This permits the same bath of periodic acid to be used many times to perform this oxidation. The periodate ion selectively oxidizes the 1-2 glycol grouping of the second and third carbon atoms and breaks the chemical bond between them. The starch chain is not ruptured in this process, so that the resulting compound is a polymeric dialdehyde structure, which gives this material its unique properties. (4,5)

Wheat gluten can be separated (6) merely by thorough kneading (or similar physical manipulation) of flour dough under a stream of water. As isolated by this "washing-out" procedure, gluten has an average water content of about 65%, while its dry substance usually contains 75-80% protein, 5-10% residual carbohydrates (chiefly starch residues), 5-10% lipids, and small quantity of mineral salts. By use of exceedingly vigorous and prolonged mechanical treatment during the washing operation, crude glutens containing 85-90% proteins (dry basis) can be obtained.

Purified gluten has been prepared by Jones *et al* in the following way (7). Flour (12% moisture) was extracted with n-butyl alcohol to remove lipids. It was then kneaded in a stream of 0.1% NaCl solution to remove starch and other non-gluten materials. The gluten ball was dispersed at 5% protein concentration in 0.01N acetic acid, centrifuged;

heated to 98-100°C (to inactivate proteolytic enzymes) and then freeze-dried. White fluffy product contained traces of acetic acid, which made it readily soluble in water. Gluten so obtained contained less than 1% lipid, less that 1% carbohydrate, 0.4% ash, 0.13% phosphorous impurities.

Proteins of the wheat gluten complex are primarily prolamines (gliadin fraction) and glutelins (glutenin fraction). (8,9) The two fractions account for about 80% of the endosperm protein in the wheat kernel. Protein make up of wheat gluten has been studied extensively (10) by many workers, yet relatively little progress had been made.

Molecular weight studies (mainly sedimentation method based on an approach to equilibrium technique) have shown that glutenin - the protein that gives gluten elasticity and cohesiveness- contains basic polypeptide building blocks. Held together by disulfide - sulfhydryl bonds, this material "forms high molecular aggregates". Jones and Taylor have reported molecular weights of 2 to 3 million for the - glutenin and about 50,000 for the main gliadin components.

Another group, headed by Dr. H. C. Nielsen (11) at USDA's Northern Research Lab., Peoria, Illinois, cleaved the disulfide bonds of glutenin with performic acid. The molecular weight of the oxidized compound was around 20,000 and the compound was unexpectedly homogeneous in molecular weight. This was confirmed by cleaving the disulfide bonds to form the S-sulfonate groups for each disulfide bond. Moving boundary electrophoresis of this degradation product yielded a pattern with a single peak. Thus by cleaving the disulfide bridges, one obtains an apparently

homogeneous species with a molecular weight of 20,000 plus loss of elasticity and cohesiveness.

Gluten can thus be considered to be a macromolecular compound containing basic polypeptide units with a molecular weight of 20,000 and various numbers of these units are linked by disulfide bonds; the elastic and cohesive properties of glutenin, according to Nielsen et al depends upon the cross linking phenomena.

It is needless to explain that the reaction of these two polymeric substances viz. gluten and DAS could be expected to yield a polymer of higher molecular weight unless the pre-experimental conditions are such that extensive chain rupture could occur in the parent polymers. The product, thus, could be called as a copolymer of gluten and DAS. Now the question is, could this copolymer be "tailor-made" like several other copolymers and useful in some way? In order to analyze this, we will break this question into two parts (1) When do we need a "tailor-made" copolymer? and (2) Is that subject relevant to gluten-DAS system?

Well, we need a "tailor-made" copolymer when we find that the reactants (or the parent polymers) have different distinct properties but when coupled together in right proportions, they yield a copolymer with unique desirable properties, which could be controlled (hence the word "tailored") by the amounts of reactants allowed to react. In fact, the reaction scheme of gluten-DAS system has been undertaken to synthesize a so called tailor-made copolymer, where the DAS will reduce some of the elastic and cohesive properties of gluten. Whether the product

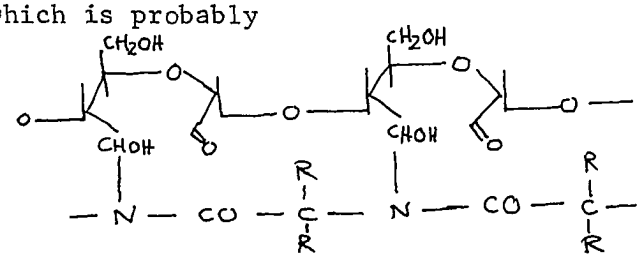
would be a plastic, a film, a fiber, an adhesive or rubber, would depend upon (1) the proportions of the parent polymers present in the reaction product and (2) the steric configuration (attactic, isotactic or syndiotactic) of the product macromolecules.

Since the configuration of gluten itself is yet to be known, emphasis has been given mainly on the ratio of the reactants. Subsequent study of the G-D product* is interesting.

*G-D product is the abbreviated form of gluten dialdehyde starch product.

PREVIOUS WORK

It was known for a long time that formaldehyde and certain other aldehydes are active tanning agents. The reactions of collagens with formaldehyde, acrolein, crotonaldehyde and glyoxal have been exhaustively studied by different workers. Recently, the commercial availability of certain polyfunctional aldehydes has created considerable importance regarding their use as tanning agents (i.e. reactions with proteins). The first bold venture that dialdehyde starches could be used to crosslink protein molecules, was made by Fein et al (12). This group found that DAS would tan calfskin. Continuing this work, the same group showed that DAS under alkaline conditions produced a leather which is comparable to other aldehyde treated leather. It is supposed that the aldehydic groups and peptide linkages react in the same fashion as in an addition polymerization process, to give a product which is probably



The information regarding this mechanism is scanty. Sloan et al (13) found quite a few years ago that when DAS was treated with urea, only one mole of urea per repeating unit reacted, leaving one carbonyl group free. These authors didn't try to locate which one of the two carbonyl functions was involved in the reaction, but on the basis of

the earlier work of Jayme and Maris (14), that one of the two aldehyde groups of periodate oxidized cellulose (that on C atom 3) has considerably greater reactivity than the other, they conjectured that one of the carbonyl groups is available for the reaction while the other is left in the hydrated or lactol ring form.

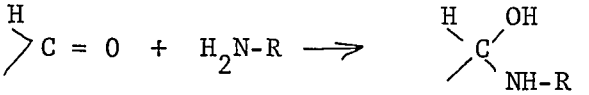
The next pioneering effort in this field was due to Weakley, Mehltretter and Rist (15). They reacted DAS with casein in aqueous borax dispersions under various conditions of pH and concentration to produce complexes having irreversible insolubility in water. Maximum combining capacity of DAS was found to be 25 grams per 100 grams of casein. Viscosity stability of DAS-casein dispersions was achieved by appropriate combinations of reagents under slightly acidic conditions. Rapid reaction and gelation occurred at pH levels above 7.

Nayudamma, Joseph and Bose (16) made a study on the combination of DAS with collagen with special reference to the effects of pH, salts, temperature, modification of collagen, modification of DAS, and pretanning and retanning with other tanning agents. Free carbonyl groups in DAS-tanned collagen were detected and estimated at different pH levels. They investigated the nature of the free carbonyl groups by degradation studies of DAS tanned collagen. They found that free carbonyl groups in the sample tanned at alkaline pH to be much lower that those in the samples tanned at neutral or acidic pH. This may be due to the fact that, the free carbonyl groups are modified in the alkaline condition.

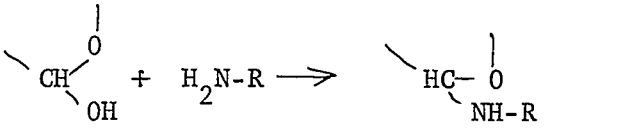
The presence of free carbonyl groups in DAS-tanned collagen was explained on the basis of complex equilibria that might be expected to exist in aqueous solutions of dialdehyde starch. They also found from the results of degradation studies of periodate oxystarch-tanned collagen that the reaction does not take place exclusively with one aldehyde group. However, the free carbonyl groups detected in DAS-tanned collagen may also arise from such dicarbonyl glucose residues which contain both the carbonyl groups free. Nayudamma *et al* (16) also studied the reactions of DAS with amino acids in order to understand the reaction mechanism. Lysine was most reactive towards DAS; mixtures of dialdehyde starches with arginine, leucine, valine, glutamic acid, asparagine and glutamine also reacted and gave brown colors of moderate intensity. The hydroxy amino acids, serine, threonine and hydroxyproline reacted much less rapidly than the other amino acids. The same trend of results was also obtained by other workers in the case of glucose (17), acetaldehyde (18) and dihydroxyacetone (19).

Nayudamma *et al* (16) did some work on infrared spectrophotometry on the reaction products of DAS and amino acids. The reaction of DAS with glycine can take place in several possible ways. The aldehyde group may react with the amino group either like a Schiff base type (I) or aldehyde-amine addition type (II). Besides, the hemiacetal and hemialdal units may also react in a similar fashion as the formation of aldosylamines.

$$\begin{matrix} H \\ \diagup \\ \diagdown \end{matrix}\!\!>C = O + H_2N\text{-}R \longrightarrow \quad \begin{matrix} H \\ \diagup \\ \diagdown \end{matrix}\!\!>C = N\text{-}R \qquad \text{(I)}$$



(II)



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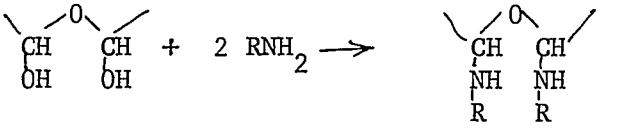

(IV)

They found a sharp peak at 6.05μ for the IR spectra of the glycine-DAS compound, which is usually caused by $\rangle C = N-$ linkage and thus it was an evidence for the structure (I). The compound also exihibited a band at 2.88μ which can be due to either an -NH or -OH grouping.

Mester (20) observed that the phenyl hydrazine derivative of DAS gave the formazan reaction, thus showing that it has a Schiff base structure. Using Bremner and Kenten's method (21), Nayudamma et al (16) found that the reaction product of DAS and glycine gave a positive reaction with ninhydrin. Ninhydrin is an effective reagent for amino acids and primary and secondary aliphatic amines. A positive reaction with this reagent, in conjunction with other observations therefore suggest that glycine-DAS compound may also contain some of the aldosylamine or aldehydeamine addition type of linkages.

The reaction between the complex proteins of wheat gluten and DAS could be expected to be very very complicated in comparison to the work with amino acid models. However, it appears reasonable to assume that, in the reaction scheme of gluten and DAS, the carbonyl groups and the nitrogen of peptide bonds will be involved in the reaction.

EXPERIMENTAL STUDIES AND RESULTS

The experimental work for the study of the reactions of gluten and DAS can be classed into three sections in a broad sense:

I. Synthesis of the G-D product*

II. Study of the G-D adhesive

III. Study of the reaction fundamentals.

Part I. Synthesis of the G-D Product

As it has been mentioned before, the first problem in reacting gluten and DAS was to find a suitable solvent system to carry out the reactions. Also high temperatures could not be used because gluten is denatured above 60°C.

The reactions of gluten and DAS were carried out by using the following different solvent combinations:

	Gluten	DAS
1.	Sodium hydroxide	Sodium hydroxide
2.	Sodium hydroxide	Borax
3.	Sodium hydroxide	Sodium acetate
4.	Acetic acid	Sodium hydroxide
5.	Acetic acid	Borax
6.	Acetic acid	Sodium acetate
7.	Acetic acid	Sodium Bisulfite
8.	Monoethanolamine	Sodium Bisulfite

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*G-D product is the abbreviated form of gluten dialdehyde starch reaction product.

The reason for selecting the solvent combinations 1 through 6 was due to previous solubility studies on gluten and DAS (22,23) and for 7 through 8 was to utilize the fundamental property (i.e. bisulfite addition reaction) of aldehyde groups.

In general, the reactions were fast as could be observed in those cases where solid products were obtained and there had been several occasions where the reactions ran out of control (i.e. so fast that, temperature, pH etc. could not be maintained).

Combination 1 Gluten in NaOH (0.1% Solution in H_2O)

93% DAS in NaOH (0.1% Solution in H_2O)

Gluten was dissolved in 0.1% NaOH solution at different concentrations at 30°C using a xonstant bath. As will be observed in Table 1, the amount of gluten used in every run as well as the amount of the solvent used are both reported. The DAS solution was made in large quantity (20 gm 93% DAS/100 ml 0.1% NaOH solution). The temperature was 30°C and solution time 2 hours. Five different amounts of DAS solution were reacted with each gluten solution; the idea was to vary the gluten : DAS ratio and (even at the same ratio) the concentration of the gluten solution.

The reactions between gluten and DAS were carried out in a Waring Blendor at 55°C (by circulating hot water from a thermostat). The pH was maintained at 8.0. The reaction time used was 15 minutes. In this series the only adhesive tests made were made with the liquid reaction product. Tensile strengths were measured to test for the adhesive property using wooden blocks of 1 square inch surface area and the testing machine described in the Appendix. Wooden blocks

glued with this liquid reaction product were kept overnight under pressure in a Carver press at 2000 p.s.i. The tensile strengths were, in general, low and showed no definite pattern.

Protein analysis was made on the precipitated (by adding 20% NaOH solution drop by drop) and dried (in vacuum drier at 60°C.) solid product or, in the cases where there was no liquid product, on the dried solid product. It was observed that the protein percentages showed no definite pattern, but the precipitated products generally had a higher protein composition. The percent protein was estimated by using the standard conversion factor of 6.25 on the percent Kjeldahl nitrogen.

The viscosities of the liquid products were evaluated on a Brookfield viscometer. "Precipitation pH" indicates the pH at which precipitate appears in the liquid product when a 20% NaOH solution is added drop by drop to the former.

Figure 1 was drawn from data in Table I showing the relation between DAS added and dissolved.

Combination 2 Gluten in 0.5% NaOH Solution

93% DAS in 1.0% Borax Solution

The gluten solutions were made exactly in the same manner as in the previous combination with one exception that a 0.5% reagent solution was used instead of a 0.1% NaOH solution. The DAS solution data are shown in Figure 2. Solution time was 2 hours and the temperature used was 30°C.

Table 1. Gluten-DAS Reaction Product, Using Sodium Hydroxide as the Common Solvent. (Combination 1) Temperature 55°C. pH 8.0

Run no.	Gms. gluten	Ml. 0.1% NaOH	Ml. DAS sln.[a]	Ratio gms. gluten/ gms. DAS	Liquid Product	Precipitation pH	Viscosity cp.	Tensile strength psi	Protein %
1	30	90	150	1-1	No	-	-	-	79.4
2	30	150	100	3-2	No	-	-	-	72.3
3	30	210	50	3-1	Yes	5.8	10.0	140	89.7
4	30	265	5	30-1	Yes	5.5	10.0	245	88.7
5	30	270	0		Yes	no ppt.	7.0	175	89.7
6	20	100	150	2-3	No	-	-	-	79.6
7	20	160	100	1-1	No	-	-	-	76.4
8	20	220	50	2-1	Yes	5.6	6.5	236	88.0
9	20	275	5	20-1	Yes	6.3	5.0	114	86.3
10	20	280	0		Yes	6.0	10.0	0	88.3
11	10	110	150	1-3	Yes	5.1	4.5	0	68.5
12	10	170	100	1-2	Yes	5.5	3.5	0	73.1
13	10	230	50	1-1	Yes	5.6	4.0	15	84.5
14	10	285	5	10-1	Yes	5.4	2.5	48	91.8
15	10	290	0		Yes	no ppt.	2.5	0	74.0
16	5	115	150	1-6	Yes	4.9		0	85.4
17	5	175	100	1-4	Yes	4.9	4.0	0	62.5
18	5	235	50	1-2	Yes	5.2	3.0	0	73.2
19	5	290	5	5-1	Yes	2.0	3.0	0	

[a] 20 gr. DAS in 100 ml 0.1/NaOH solution.

Table 1. (Continued)

Run no.	Gms. gluten	Ml. 0.1% NaOH	Ml. DAS sln.[a]	Ratio gms. gluten/ gms. DAS	Liquid Product	Precipitation pH	Viscosity cp.	Tensile strength psi	Protein %
20	5	295	0		Yes	no.ppt.	3.0	0	
21	15	105	150	1-2	No	-	-	-	90.5
22	15	165	100	3-4	Yes	5.6	4.5	0	48.3
23	15	225	50	3-2	Yes	5.5	5.5	43	86.0
24	15	280	5	15-1	Yes	4.8	4.5	87	90.0
25	15	285	0		Yes	no ppt.	4.0	0	

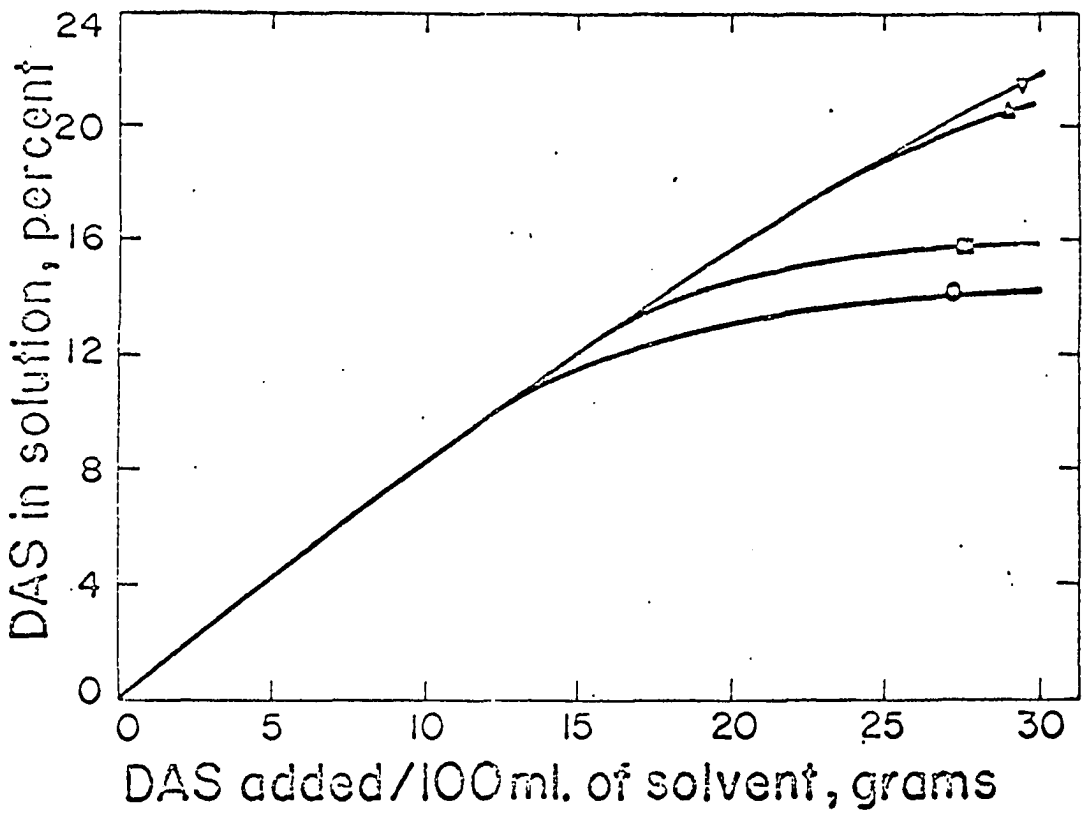

Figure 1. Amount of 93% dialdehyde starch dispersed for different time periods in different concentrations of sodium hydroxide solution as the solvent. (22, 23)

○ 15 min. 0.05% NaOH
□ 30 min., 60 min., 120 min. 0.05% NaOH
△ 15 min., 30 min., 60 min. 0.1% NaOH
▽ 120 min. 0.1% NaOH

The reaction conditions were all same as in combination 1. The product obtained was very viscous; it is believed that DAS is present in longer chains in the borax solutions than in the NaOH solutions. The longer chains of DAS probably caused the reaction product of much higher molecular weight and highly complicated structure thus rendering some of these runs completely uncontrolled. With the blendor operating at full power there was very little mixing in the upper portion of the blending jar thus resulting in a product of questionable uniformity. This also made it impossible to control the pH in this combination and use of the temperature control coil in some of these runs. Finally the product obtained was in the state of complete gel. The adhesive test was carried out on the alcohol dried product (which was cream colored powder) using 0.5% NaOH solution as the solvent. Results are given in Table 2.

Combination 3 Gluten in 0.5% NaOH Solution
93% DAS in 5% NaOAc Solution

Gluten solutions were made in the same fashion as in Combination 2. 93% DAS was dissolved in 5% sodium acetate solution at 30°C, using a solution time of 1 hour (vide Figure 3). The stock solution contained 21.6 gms DAS per 100 ml sodium acetate solution.

The reaction conditions were the same as in the previous combinations. In most cases, the product formed into a gel. Most of the runs were controllable although a few runs with high gluten concentrations were not. Wherever control was possible, the temperature was maintained at 55°C. and the pH at 8. In this series, the adhesive tests

Table 2 Gluten-DAS Reaction Product. Gluten Dissolved in NaOH Solution, DAS in Borax Solution. (Combination 2)

Run no.	Gms. gluten	Ml. 0.5% NaOH	Ml. DAS sln.[a]	Ratio gms gluten/ gms DAS	Adhesive mixture gm/ml .5% NaOH	Tensile strength psi	Protein %
30	60	200	4	60-1	2/25	65	78.4
31	40	200	111	4-3	2/50	70	62.8
32	40	200	75	2-1	2/30	107	70.0
33	40	200	37	4-1	2/30	236	73.7
34	40	200	19	8-1	2/50	162	76.4
35	40	200	4	40-1	2/25	65	80.3
36	40	160	93	8-5	2/45	228	67.2
37	45	180	75	9-4	2/35	175	66.1
38	50	200	37	5-1	2/50	187	74.7
39	40	160	19	8-1	2/40	140	79.7
40	50	200	4	50-1	2/45	490	68.9
41	30	200	93	6-5	2/30	162	65.5
42	30	200	75	3-2	2/40	122	66.3
43	30	200	37	3-1	2/50	81	64.3
44	30	200	19	6-1	2/30	35	51.8
45	30	200	4	30-1	2/35	74	80.2

[a] 26 grams DAS in 100 ml 1.0% borax solution.

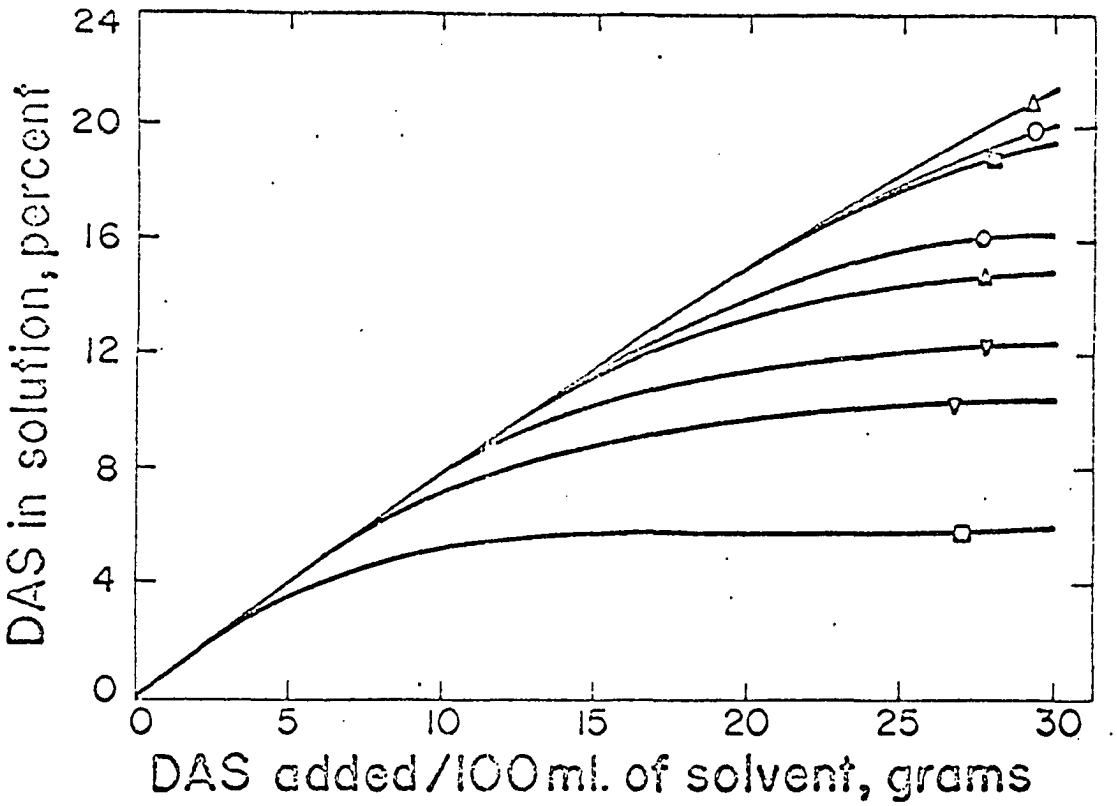

Figure 2. Amount of 93% dialdehyde starch dispersed for different time periods in different concentrations of borax solution as the solvent. (22, 23)

▽ 15 min. 1.05 Borax
○ 30 min. 1.0% Borax
□ 60 min. 1.0% Borax
○ 120 min. 1.0% Borax; 15 min., 30 min., 60 min., 5.0% Borax
△ 60 min. 0.5% Borax
▽ 30 min. 0.5% Borax
□ 15 min. 0.5% Borax
△ 120 min. 5.0% Borax

were also made on the alcohol dried product; 2 gms of solid was dissolved in 30 ml of 0.5% NaOH and the viscous paste was tested for adhesive property (tensile strength) in the same way as in the previous combinations. Results are given in Table 3.

Combination 4 Gluten in 2% acetic acid

93% DAS in 0.1% NaOH

The gluten solution was prepared in a Waring blendor at 55°C (this temperature was selected arbitrarily; gluten is denatured at 60°C); solution time was 20 minutes. Solution methods for DAS are the same as in Combination 1.

The reactions were carried out at 55°C and pH 3.5 using the general procedure, used in previous combinations. With the exception of runs 66 through 69, where the product was a mixture of a gel and a liquid, all of the products were liquid. Foaming was controlled by a silicone anti-foaming agent, "Dow-Corning Antifoam A Spray". The adhesive tests were carried out on the liquid products or where a gel was obtained, on glacial acetic acid solution (2 gm/10 ml gl. acetic acid) of the hydrophobic gel which has been previously washed with water. Adhesive strengths varied from 0 to 424 p.s.i. Protein analyses were made on the precipitated and alcohol-dried products or, in the case of no liquid product, on the dried solid product. Protein percentages did not vary greatly. The viscosity of the liquid products varied from 4 to 180 cp depending upon the ratio of the ingredients. The solid products were soluble in 5% sodium hydroxide solution, softened in 5% HCl and in distilled water, and were not affected by commercial hexane (Skelly solve B). Results are given in Table 4.

Table 3 Gluten-DAS Reaction Products. Gluten Dissolved in NaOH Solution, DAS in Sodium Acetate Solution (Combination 3)

Run no.	Gms. gluten	NaOH Ml. 0.5%	Ml. DAS sln.[a]	Ratio gms. gluten/ gms. DAS	Tensile strength psi	Protein %	pH	Temp. °C.	Gel
46	37.5	150	115	7-5	315	69.9	8	U.C.[b]	
47	40	160	92	2-1	236	70.5	U.C.	55	
48	50	200	46	5-1	170	75.1	U.C.	U.C.	
49	50	200	23	10-1	109	77.2	U.C.	U.C.	Yes
50	50	200	4.5	50-1	188	80.1	U.C.	U.C.	Yes
51	30	150	115	6-5	0	71.4	8	55	
52	35	175	83	2-1	153	71.3	8	55	
53	40	200	46	4-1	323.7	76.8	U.C.	55	
54	40	200	23	8-1	35	75.1	U.C.	U.C.	Yes
55	50	250	4.5	50-1	157.5	80.1	8	55	Yes
56	25.5	170	115	1-1	100	71.6	8	55	
57	27	180	92	9-7	354	77.2	8	55	
58	30	200	46	3-1	305	53.3	8	55	Yes
59	30	200	23	6-1	70	79.0	8	U.C.	
60	35	235	4.5	35-1	180	77.3	8	55	

[a] 21.6 grams DAS in 100 ml sodium acetate solution.

[b] U.C. - uncontrolled run.

Table 3. (Continued)

Run no.	Gms. gluten	Ml. 0.5% NaOH	Ml. DAS sln.[a]	Ratio gms. gluten gms. DAS	Tensile strength psi	Protein %	pH	Temp. °C.	Gel
61	18	180	115	3-4	122.5	69.4	8	55	
62	20	200	92	1-1	516.1	73.0	8	55	
63	20	200	46	2-1	258	77.5	8	55	
64	20	200	23	4-1	258	77.5	8	55	
65	27	270	4.5	27.1	196.8	81.7	8	55	

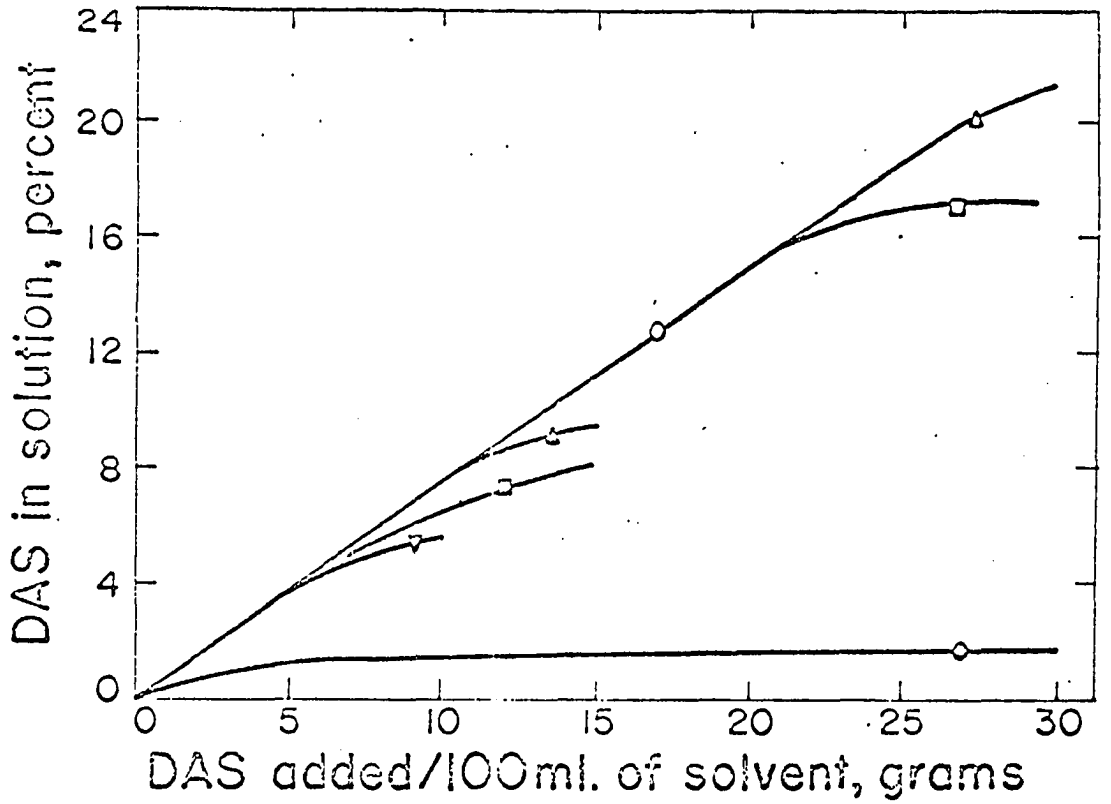

Figure 3. Amount of 93% dialdehyde starch dispersed for different time periods in different concentrations of sodium acetate solution as the solvent. (22, 23)

○	30 min. 0.5% NaAc; 15 min. 1.0% NaAc
▽	30 min. 1.0% NaAc
□	15 min. 5.0% NaAc
△	60 min. 1.0% NaAc
□	120 min. 1.0% NaAc
○	30 min. 5.0% NaAc
∠	60 min., 120 min. 5.0% NaAc

Table 4 Gluten Dissolved in 2% Acetic Acid, DAS in 0.1% Sodium Hydroxide Temperature 55°C, pH 3.5 (Combination 4)

Run no.	Gluten gm/100 ml acetic acid	Gluten Ml Soln.	DAS gr.[a]	Consistency[b]	Iso-electric point pH	Adhesive Strength psi	Protein %
66	25	170	26	G	-	394	68.9
67	25	180	12	G	-	70	73.3
68	25	200	6	G	-	-	76.4
69	25	200	2	L	-	44	74.0
70	25	200	1	L	-	153	81.5
71	20	170	25	L	-	53	74.2
72	20	180	12	L	4.0	88	63.0
73	20	200	6	L	3.4	92	69.2
74	20	200	2	L	4.1	101	79.8
75	20	200	1	L	4.4	74	63.2
76	15	180	25	L	4.7	302	-
77	15	180	12	L	4.0	26	72.1
78	15	200	6	L	4.1	333	62.3
79	15	200	2	L	4.1	193	76.1
80	15	200	1	L	4.1	0	79.8

[a] 14.7 gms DAS in 100 ml of solution

[b] G, gel; L, liquid

Table 4. (Continued)

Run No.	Gluten gm/100 ml acetic acid	Gluten Ml Soln.	DAS gr.[a]	Consistency[b]	Iso-electric point pH	Adhesive Strength psi	Protein %
81	10	180	25	L	4.2	18	65.5
82	10	180	12	L	4.2	424	66.9
83	10	200	6	L	3.9	92	75.2
84	10	200	2	L	4.4	0	77.5
85	10	200	1	L	4.1	66	79.5
86	5	180	25	L	4.1	0	59.3
87	5	180	12	L	4.2	0	70.3
88	5	200	6	L	3.9	136	75.3
89	5	200	2	L	4.8	214	79.3
90	5	200	1	L	4.3	201	-

Combination 5 Gluten in 5% acetic acid

93% DAS in 1% borax solution

Gluten solution was made in 5% acetic acid; the procedure was the same as Combination 4. The DAS solution was made in the same fashion as in Combination 2.

Reactions were carried out in a Waring blendor. Reactions involving 25% gluten solution resulted in a product which was such a thick gel even at room temperature that they were dropped. The reactions involving 20% gluten were run at 26°C. All other concentrations were attempted at 55°C. As was suspected in the Combination 2, here also it may be possible that DAS probably exists in longer chains in borax solution, thus resulting in greater gel formation. Adhesive tests of this series were as high as 543 p.s.i. (Table 5). Solubility characteristics of the solid products were similar to those in Combination 4.

Combination 6 Gluten in 5% acetic acid

93% DAS in 5% sodium acetate

Gluten solutions were prepared in the same way as in Combination 5. DAS solution was prepared as in Combination 3.

The reactions of this combination were so rapid as to be difficult to control. The typical products were mostly gummy, (some of which were unsoluble) separating out with a supernatant liquid varying from clear to yellow. Portions of solid products were dissolved in glacial acetic acid (2 gms per 10 ml HOAc) and tested for adhesive strength. Liquid products were tested directly as adhesives. Results are given in Table 6.

Table 5 Gluten-DAS Reaction Products. Gluten Dissolved in 5% Acetic Acid, DAS in 1% Borax (Combination 5)

Run No.	Gluten gm/100 ml acetic acid	Gluten Ml Soln.	DAS gr.[a]	Consistency[b]	Adhesive Strength psi	Protein %
96	20	90	12.5	L	153	71.1
97	20	90	6.0	L	494	78.5
98	20	100	3.0	L	494	87.3
99	20	100	1.0	L	556	94.2
100	20	100	0.5	L	543	94.6
101	15	180	25.0	G	-	-
102	15	180	12.0	G	-	-
103	15	200	6.0	L	9	87.5
104	15	200	2.0	L	118	74.7
105	15	200	1.0	L	0	96.3
106	10	180	25.0	L	66	48.8
107	10	180	12.0	L	162	76.8
108	10	200	6.0	L	0	88.2
109	10	200	2.0	L	35	95.7
110	10	200	1.0	L	66	97.0

[a] 21.5 gms DAS in 100 ml solution.

[b] L, liquid; G, gel.

Note: Runs 91 through 96 were exceedingly thick and discarded as unworkable.

Table 5. (Continued)

Run No.	Gluten gm/100 ml acetic acid	Gluten Ml Soln.	DAS gr.[a]	Consistency[b]	Adhesive Strength psi	Protein %
111	5	180	25.0	L	0	50.9
112	5	180	12.0	L	140	61.3
113	5	200	6.0	L	127	80.4
114	5	200	2.0	L	96	88.8
115	5	200	1.0	L	22	90.3

Table 6. Gluten-DAS Reaction Products. Gluten Dissolved in 5% Acid, DAS in 5% Sodium Acetate Temperature 55°C. Time 15 minutes (Combination 6)

Run no.	Gluten gm/100 ml acetic acid	Gluten Ml Soln.	DAS gr.[a]	Consistency[b]	Adhesive Strength psi	Protein %
116	20	180	25	G	-	88.0
117	20	180	12	G	-	84.5
118	20	200	6	G	392	93.3
119	20	200	2	G	-	93.6
120	20	200	1	L	223	95.5
121	15	180	25	G	-	71.0
122	15	180	12	G	-	80.7
123	15	200	6	G	-	90.0
124	15	200	2	G	385	93.5
125	15	193	1	L	236	95.8
126	10	180	25	G	-	85.3
127	10	180	12	G	-	80.8
128	10	180	6	G	-	95.0
129	10	180	2	L	471	97.5
130	10	180	1	L	438	99.2

[a] 17.4 gms DAS in 100 ml solution.

[b] L, liquid; G, gel.

Table 6. (Continued)

Run no.	Gluten: gm/100 ml acetic acid	Gluten: Ml Soln.	DAS gr.[a]	Consistency[b]	Adhesive Strength psi	Protein %
131	5	180	25	G	-	77.0
132	5	180	12	G	-	85.0
133	5	200	6	G	499	92.4
134	5	200	2	L	486	100.8
135	5	200	1	L	140	101.2

Combination 7 Gluten in 5% acetic acid

93% DAS in 5% $NaHSO_3$

The runs of this combination were carried out in duplicate; one set at 26°C. and the other at 55°C. Reaction time was 10 minutes each. The products from all except nine of these runs, which were liquids, were mixtures of gels and liquids. No foaming problems were encountered. In most cases, the solid fraction separated immediately from the liquid. The final adhesives for testing were produced by dissolving the gels in glacial acetic acid (2 gms/10 ml gl. HOAc). The solid products were slowly soluble in 5% sodium hydroxide. They softened in 5% HCl and in distilled water but were unaffected by commercial hexane (Skelly solve - B).

The adhesive strengths ranged from 70 to 787 p.s.i. with the common values from 400 to 600 p.s.i. When the product testing 787 p.s.i. was retested a value of 971 was secured. For comparable reactant ratios, the reactions at room temperature produced better products than those at 55°C. Data for this combination are shown in Tables 7a and 7b.

Combination 8 Gluten in 1% monoethanolamine

93% DAS in 5% $NaHSO_3$

One percent monoethanolamine in water was used as the solvent for gluten. Gluten solutions were prepared at 26°C. Attempts to produce a 20% gluten solution in monoethanolamine were unsuccessful. Three gluten concentrations were used: 5, 10 and 15 gms per 100 ml. All reactions were carried out at 26°C. Reaction time was 10 minutes.

Table 7a. Gluten - DAS Reaction Products Gluten in 5% acetic acid. DAS in 5% sodium bisulfate. Temperature 55°C. Time 10 minutes (Combination 7)

Run no.	Gluten[a] g.	DAS Solution DAS g.	DAS Solution ml. $NaHSO_3$ solution[b]	Reaction Product Type[c]	Reaction Product Adhesive Strength psi	Reaction Product Protein %
136	40	25	500	G	538	----
137	40	12	250	G	604	88.3
138	40	6	150	G	752	86.2
139	40	2	75	G	521	86.0
140	40	1	50	G	380	90.6
141	30	25	500	L	499	26.8
142	30	12	250	L	298	72.0
143	30	6	150	L	441	78.0
144	30	2	75	G	197	78.8
145	30	1	50	G	477	93.7
146	20	25	500	G	372	17.5
147	20	12	250	G	429	88.0
148	20	6	150	G	516	79.0
149	20	2	75	G	298	84.8
150	20	1	50	G	547	92.3

[a] In 200 ml. 5% acetic acid in distilled water.

[b] 5% $NaHSO_3$ in distilled water.

[c] G, gel; L, liquid.

Table 7a. (Continued)

Run no.	Gluten[a] g.	DAS Solution DAS g.	DAS Solution ml. $NaHSO_3$ solution[b]	Reaction Product Type[c]	Reaction Product Adhesive Strength psi	Reaction Product Protein %
151	10	25	500	G	70	16.7
152	10	12	250	G	219	43.4
153	10	6	150	G	101	90.6
154	10	2	75	G	459	85.3
155	10	1	50	G	286	89.2

Table 7b. Gluten - DAS Reaction Products Gluten in 5% acetic acid. DAS in sodium bisulfite. Temperature 26°C. Time 10 minutes (Combination 7)

Run no.	Gluten[a] g.	DAS Solution DAS g.	DAS Solution ml. $NaHSO_3$ solution[b]	Type[c]	Adhesive Strength tensile psi	Protein %
136A	40	25	500	L	503	70.6
137A	40	12	250	L	639	52.9
138A	40	6	150	L	599	81.5
139A	40	2	75	G	788	83.2
140A	40	1	50	G	661	78.9
141A	30	25	500	L	481	82.8
142A	30	12	250	L	359	79.2
143A	30	6	150	L	464	83.2
144A	30	2	75	G	324	92.8
145A	30	1	50	G	325	94.5
146A	20	25	500	G	521	85.8
147A	20	12	250	G	275	90.0
148A	20	6	150	G	462	91.2
149A	20	2	75	G	105	83.5
150A	20	1	50	G	265	92.0

[a] In 200 ml. 5% acetic acid in distilled water.

[b] 5% $NaHSO_3$ Solution in distilled water.

[c] G, gel; L, liquid.

Table 7b. (Continued)

Run no.	Gluten[a] g.	DAS g.	Solution ml. $NaHSO_3$ solution[b]	Type[c]	Adhesive Strength tensile psi	Protein %
151A	10	25	500	G	170	85.5
152A	10	12	250	G	293	31.8
153A	10	6	150	G	105	48.4
154A	10	2	75	G	341	85.7
155A	10	1	50	G	127	82.6

Solubility characteristics of the products were similar to those of the preceeding series. Adhesive strengths (table 8) ranged from 380 to 774 p.s.i.

Ageing Studies on the Reactants

This investigation was undertaken to see if the ageing of the gluten and/or DAS solution (for run #139A) would produce a better or poorer reaction product than that made from unaged materials. These experiments were arranged in such a fashion that the results could be analyzed statistically and conclusions could be made as to:

1. Whether or not there was any effect of ageing. If there was any effect, then complete analysis on gluten and DAS solutions would have to be done including molecular weight (number average and weight average) studies, viscometry, and spectrum analysis.

2. If the best run #139A was taken to be a standard method of preparing G-D adhesive, then how much variance could be expected in the final strength of the glue, where the sources of variation could be broadly divided into: (a) all process conditions and (b) surface conditions.

Reaction procedure

The reaction conditions used for the products tested are those given the Table 7b for run number 139A. Two hundred ml of a 20 percent solution of gluten in 5 percent acetic acid were reacted with 75 ml of 2.66 percent solution of 93 percent DAS at 26^{o}C for 10 minutes.

Table 8. Gluten - DAS Reaction Products Gluten in 1% monoethanolamine. DAS in 5% sodium bisulfite. Temperature 26°C. Time 10 minutes (Combination 8)

DAS				Reaction Product		
Run no.	Gluten[a] g.	g.	ml. solution	Type[b]	Adhesive Strength, psi	Protein %
156	10	25	500	G	236	----
157	10	12	250	G	420	96.8
158	10	6	150	G	464	88.1
159	10	2	75	G	453	98.0
160	10	1	50	G	543	98.8
161	20	25	500	G	290	----
162	20	12	250	G	458	53.1
163	20	6	150	G	503	74.0
164	20	2	75	G	774	90.7
165	20	1	50	G	446	93.6
166	30	25	500	G	301	----
167	30	12	250	G	381	93.1
168	30	6	150	G	547	93.5
169	30	2	75	G	766	87.8
170	30	1	50	G	595	96.3

[a] In 200 ml. MEA.

[b] G, gel.

The solid product, which was rubbery, was washed with water three times, mixed with absolute alcohol and churned up in the Waring Blendor, filtered in Buchner, air dried, and bottled.

The solid was dissolved in a 5 percent slurry of calcium hydroxide in distilled water and the solution applied to maple test blocks. After curing[a] tensile strengths were determined.

Reaction pattern

Setting up the reaction pattern followed statistical methods. The symbols used are as follows: a reaction is represented in the figures by a square with superscripts 1, 2, 3, 4, and 5 representing the gluten solution streams and the corresponding subscripts representing the gluten solution streams and the corresponding subscripts representing the DAS streams. Thus, for example, $\square_2^1 \equiv$ reaction between the first stream of gluten solution and the second stream of DAS solution. The dates corresponding to each stream are shown on Figure 4 which shows the general reaction pattern.

On August 5, 1962, the reactions shown in Area B were carried out. In addition to these 16 reactions, nine others were picked up at random. These were 1/1 (or $\square_1^1$), 1/2, 1/4, 2/4, 3/1, 3/3, 4/1, 4/2, and 4/4. These are shown as solid squares in Figure 4. This gave a total of 25 reactions on August 5th.

On August 13, 1962, all reactions except those enclosed within Area A were carried out. This gave a total of 14 reactions.

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[a]Pressed in a Carver press overnight at 2000 p.s.i.

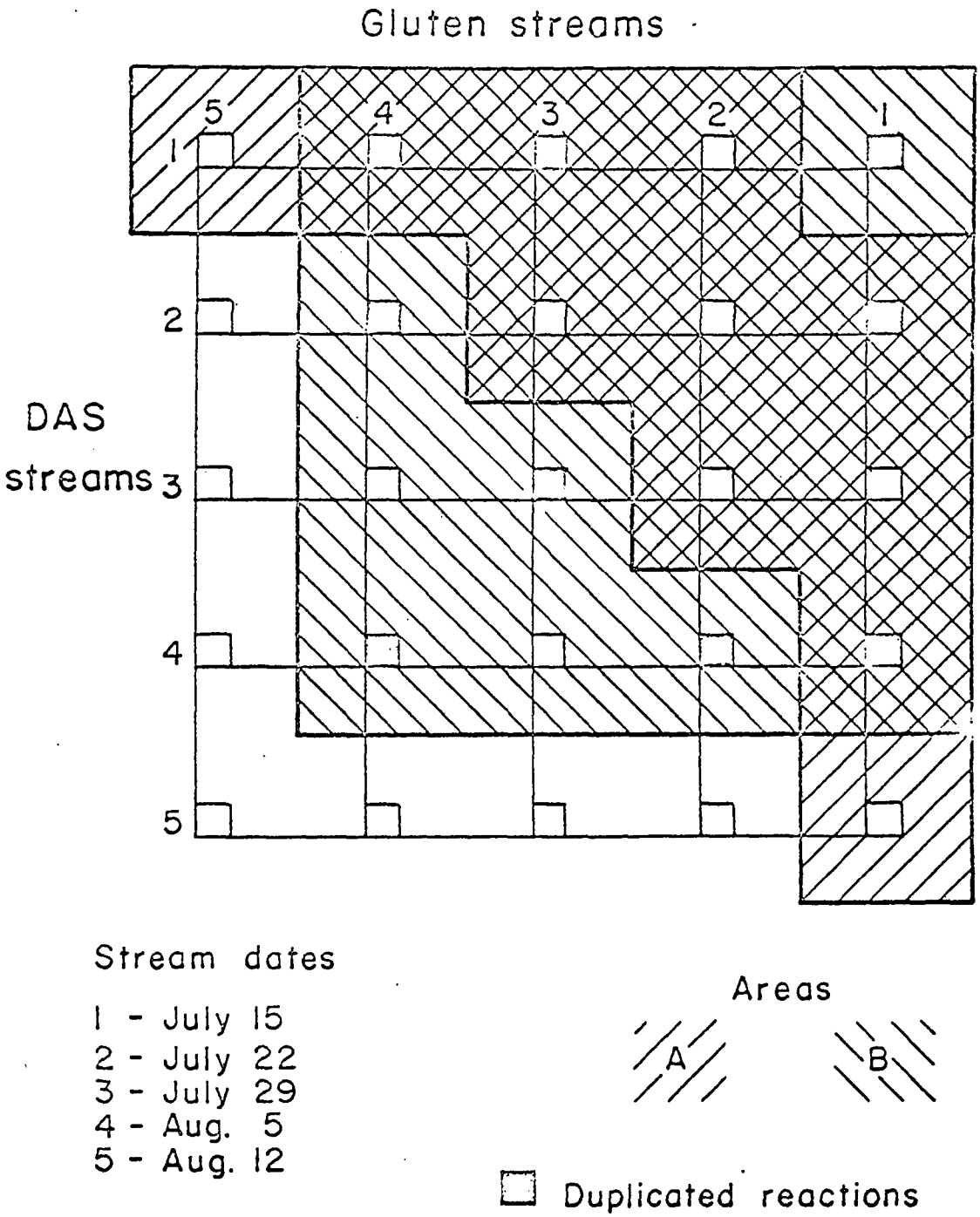


Figure 4. Reaction pattern

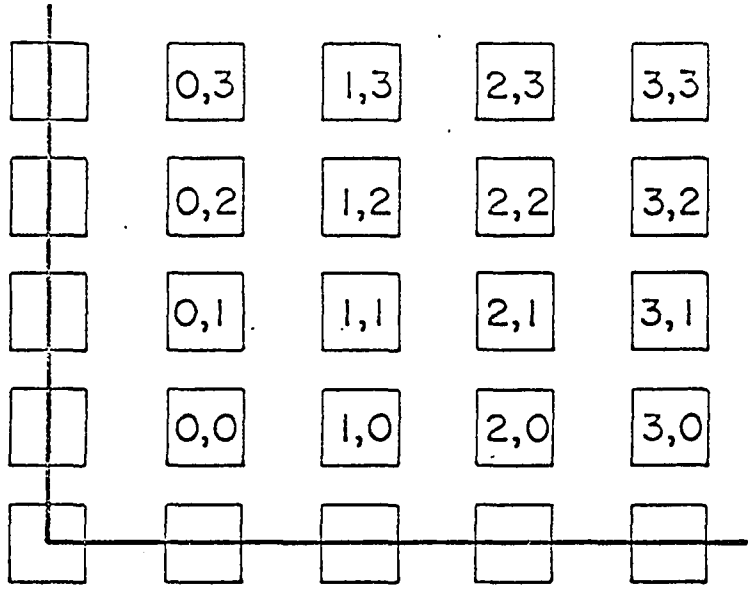
The reaction pattern used had the advantage of replicating most of the reactions. For example 4/4 of August 5 is equivalent to 5/5 of August 12. The maximum aging effect is shown with 1/1 on August 12, where both streams prepared on July 15 had aged for 5 weeks.

Results

The pattern of reaction for August 5th is shown in Figure 5. The digits within the squares represent the age in weeks of the reacting solutions, the left and right hand digits representing gluten and DAS respectively. The reactions of August 12 are shown in Figure 6.

The results are shown in Tables 10 and 11 for August 5th and August 12th respectively. Here a simplified notation is used in which the squares have been dropped. Statistically, there was no apparent correlation between the tensile strength and ageing.

The values of tensile strength (p.s.i.) which were obtained from the replicate runs and shown in Figure 7, were better results than their corresponding replicas. The conclusion is not statistically sound but was accepted on the understanding that the higher strength would correspond to better experimental conditions. Comparing only the experiments 1,1; 2,2; 3,3 and 4,4 of August 5, 1962, we can conclude that with a small sample size, such as we had, we do not have sufficient evidence to conclude that ageing has either a desirable or deleterious effect on the final strength of the glue. Therefore we are accepting the indirect evidence that ageing did not change the glue property even though we have large variations in the tensile strength.



1st and 2nd digits left to right are gluten and DAS respectively

Figure 5. Reaction situation on Aug. 5, 1962

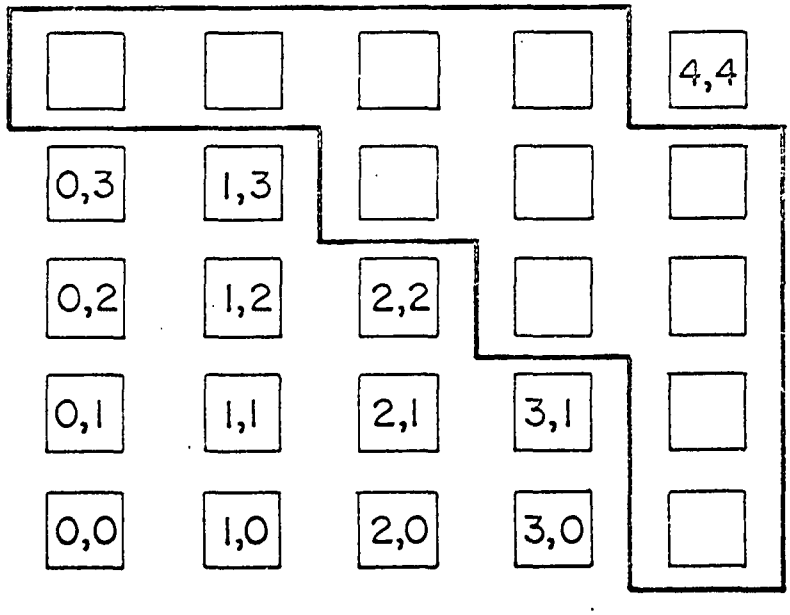


Figure 6. Reaction situation on Aug. 12, 1962

Table 9a. Tensile Strengths of Reaction Products of August 5, 1962

Reaction Streams[a]	Stream Ages in weeks[a]	Tensile Strength of glued joints lb./sq. in.
1/1	3.3	1159.0, 400
1/2	3.2	1137.5, 717.5
1/3	3.1	1181.0
1/4	3.0	774.0, 720
2/1	2.3	533.8
2/2	2.2	953.8
2/3	2.1	542.5
2/4	2.0	1154.0, 887
3/1	1.3	1094.0, 990
3/2	1.2	1137.5
3/3	1.1	1146.2, 976
3/4	1.0	560.0
4/1	0.3	520.6, 501
4/2	0.2	704.0, 553
4/3	0.1	533.8
4/4	0.0	1146.2, 1146.2

[a]Left hand digit, gluten; right hand digit, DAS. Underlined numbers indicate duplicated reactions.

Table 9b. ANOVA Table

Source	d.f.	SS	MS
Process Variation	15	1,735,462.86	115,697.52
Variation due to surface	9	44,480.15	49,422.38
Total	24		

Table 10. Tensile Strengths of Reaction Products of August 12, 1962

Reaction Streams[a]	Stream Ages in weeks[a]	Tensile Strength of glued joints lb./sq. in.
1/1	4.4	X
2/4	3.1	X
2/5	3.0	X
3/3	2.2	656.0
3/4	2.1	X
3/5	2.0	X
4/2	1.3	m/c unworkable
4/3	1.2	m/c unworkable
4/4	1.1	X
4/5	1.0	953.8
5/2	0.3	673.8
5/3	0.2	533.8
5/4	0.1	m/c unworkable
5/5	0.0	700.0

[a] X Glue failure before reaching 500 p.s.i. (any value below 500 p.s.i. has been rejected in this experiment set).

The data secured for ageing studies (Table 9) (experiments of August 5, 1962) were computed to set up the ANOVA Table (Table 9b).

The model we assumed in this analysis of variance is of the type:

$$X_{ij} = M + A_i + E_{ij}$$

Where

$$A_i \sim N(0, \sigma_A^2)$$

and $$E_{ij} \sim N(0, \sigma_E^2)$$

(i.e A_i is normally distributed with mean zero and variance σ_A^2; E_{ij} is normally distributed with mean zero and variance σ_E^2) so we have

$$AMS \sim \sigma_E^2 + N_o \sigma_A^2$$

Where N_o is given by

$$N_o = \frac{1}{a-1}\left[n_. - \frac{\sum_i n_i^2}{n_.} \right]$$

Where a = number of distinct treatment

$= 16$

$n_.$ = total number of experiments

$= 25$

$$\sum n_i^2 = 9\ (2)^2 + 7\ (1)^2$$

$$= 36 + 7$$

$$= 43$$

Hence N_o comes out to be 1.55 and $WMS \sim \sigma_E^2$

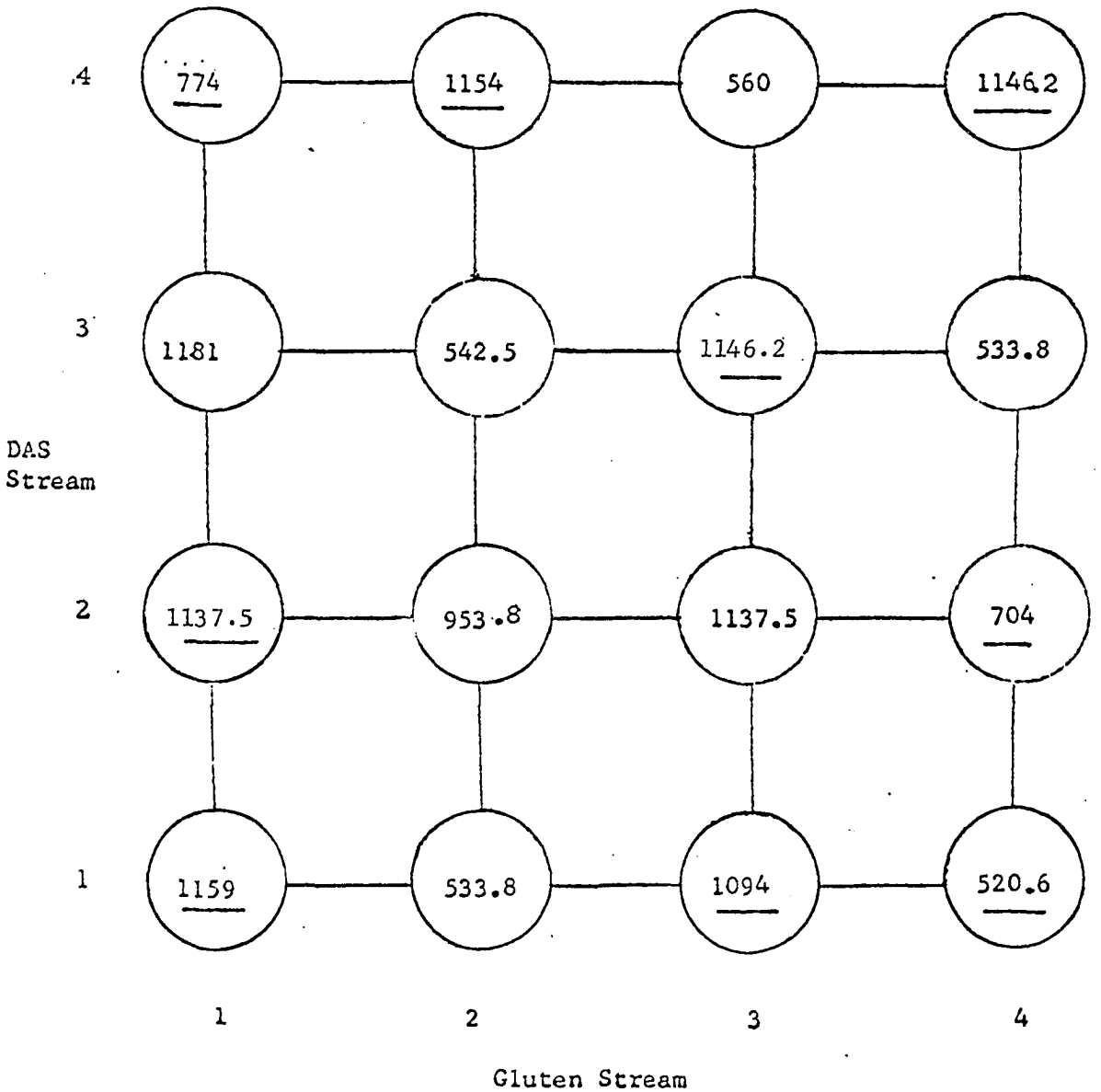


Figure 7. Best tensile strength data from the reaction products of August 5, 1962, as plotted against reactant streams.

Single experiment

Experiment repeated

$$\frac{AMS}{WMS} = 1 + 1.55 \frac{\sigma_A^2}{\sigma_E^2}$$

$$= \frac{115,697.52}{49,422.38}$$

$$= 2.34$$

$$\frac{\sigma_A^2}{\sigma_E^2} = \frac{1.34}{1.55} = 0.86 = \frac{\text{(Variance) process}}{\text{(Variance) surface}}$$

This shows that variance due to surface inhomogenity influences the final strength of the glue more than the process variation (i.e experiment error).

Also we can compute the statistic $\frac{AMS}{WMS} = 2.34$ and compare with F table at 5% significant level with degrees of freedom ν_1=15 and ν_2=9 which is 3.0.

This leads us to accept the Ho: $A_i = 0$ because we have insufficient evidence to show $A_i > 0$; in other words, we say, it is not implausible that, indeed, all the variation is attributable to surface conditions.

Part II. Study of the G-D Product

The study of the adhesive comprises the following items: 1) study of the solvent for the solid G-D product to obtain the best possible glue, 2) study of the (1) G-D product (solute) to solvent ratio, (2) mixing time, (3) curing time in the press and (4) the effect of ageing the glued joints. These experiments have been designed in graeco-latin square arrangement and the variation due to different sources are computed statistically to obtain a quantitative measure of the variance in tensile strength due to each variable.

Study of Solvents for the Solid Adhesive

The following solvents, which were first tried qualitatively and resulted in varying amounts of success, have been used for quantitative evaluation:

1. glacial acetic acid
2. calcium hydroxide slurry (in distilled water)
3. dimethyl sulfoxide
4. N-N dimethyl formamide
5. sodium hydroxide (5% in distilled water)
6. sodium bicarbonate (5% in distilled water)
7. sodium acetate (5% in distilled water)

All solvents were used with the solid product from run 139A. The results with the first five solvents are shown in Table 11. Sodium bicarbonate and acetate were not satisfactory. Glacial acetic acid, which was the first solvent used, gives good results but is objectionable from corrosion standpoint. Sodium hydroxide gave good results. The most promising results were secured with calcium hydroxide slurry.

Study of the (1) G-D Product (Solid) to Solvent (5% $Ca(OH)_2$ Slurry) Ratio; (2) Mixing Time; (3) Curing Time in the Press; and (4) The Effect of Ageing the Glued Joints

The best solvent for the G-D product, i.e. a 5% slurry of $Ca(OH)_2$ in distilled water has been identified in the Section (1) of Part II. Now in order that the solution (or paste) would be suitable as a standard adhesive, it calls for a good suspension of the G-D product macromolecules

Table 11. Comparative Solvent Results

Solid Product gms	20 ml solvent	Description of the adhesive	Tensile Strength p.s.i.
		1. Solvent: Glacial Acetic Acid	
	1	Thin liquid but little sticky.	170
	2	More sticky than the previous one; very thin like the first case.	420
	3	Thicker and more sticky liquid; faint yellow color.	437
	4	Sirupy liquid with light yellow color; appreciably sticky.	813.8
	5	Viscous liquid with light yellow color; pretty sticky.	520[a]
2. Calcium Hydroxide (20% slurry in distilled water)			
	1	Very thin; apparently not much adhesive.	323
	2	Thicker than the previous one. Little bit sticky.	643
	3	Desirable consistency, viscous, pretty much thick.	910
	4	Very thick with appreciable stickiness.	647
	5	Very thick; sticky but the solids can be felt if rubbed between fingers.	757
2b. Calcium Hydroxide (15% slurry in distilled water)			
	1	Very thin liquid with too much bubbles.	113.8
	2	Thin liquid; not much adhesive in nature.	306.2
	3	Viscous liquid; faint yellow color; sticky.	927.5
	4	Viscous liquid with faint yellow color; more sticky.	901[a]
	5	More viscous liquid; very sticky.	840
2c. Solvent: Calcium Hydroxide (10% slurry in distilled water)			
	1	Thin liquid with bubbles. Little sticky.	424

[a] Complete wood failure.

Table 11. (Continued)

Solid Product gms	20 ml solvent	Description of the adhesive	Tensile Strength p.s.i.
2		Thin liquid; sticky; faint yellow.	437
3		Sirupy liquid with faint yellow color; sticky.	542.5[a]
4		Viscous light yellow liquid; considerably sticky.	752.5
5		More viscous and light yellow liquid; very sticky.	608[b]
2d. Solvent: Calcium Hydroxide (5% slurry in distilled water)			
1		Thin liquid; sticky	485.5
2		Thin liquid but considerably sticky.	962.5
3		Thicker liquid than the previous ones; faint yellow color; very sticky.	980
4		Viscous liquid; light yellow color; very sticky.	761[a]
5		More viscous liquid; light yellow color; very sticky.	1,067.5
		3. Solvent: Dimethyl Sulfoxide	
1		Very thin solution. Some solid settles as sludge after 48 hours. Non-sticky. Color is light yellow	103.8
2		Little viscous solution. Little stickiness; oily feel.	301.9
3		Viscous solution; appreciable sticky.	393.8
4		Pretty viscous solution. More sticky, than any of the previous ones.	511.9
5		Very very viscous. Takes little longer time to go into solution completely. Sticky but difficult to handle	433
		4. Solvent: N-N Dimethyl formamide	
1		Very thin liquid; doesn't work like adhesive.	288.7

[b]Partial wood failure.

Table 11. (Continued)

Solid Product gms	20 ml solvent	Description of the adhesive	Tensile Strength p.s.i.
2		Thin oily liquid; little sticky.	393
3		Comparatively thicker consistency; sticky.	463.8
4		Viscous liquid; sticky.	284
5		More viscous liquid; sticky.	253.8
5.	Solvent: NaOH (1% solution in distilled water)		
1		Thin liquid; sticky; yellow color.	660.6[a]
2		Thin liquid; deep yellow color; sticky.	857.5[b]
3		Thin liquid; light brown color; pretty sticky.	975.5
4		Thin liquid; brown color, pretty sticky.	787.5
5		Dark brown; thin liquid; very sticky.	717.5

in the $Ca(OH)_2$ slurry as well as quick setting of the adhesive. So the solution (or paste) needs to be of proper consistency, sufficiently tacky (so that the surfaces of application don't slip away before the final curing) and still keeping the gigantic molecules in right suspension.

The time of mixing of the solid and the solvent may have some effect in the sense that for the same solid: solvent ratio, lesser time might not bring about proper suspension and more time might result in advancing the curing operation before it is applied to the surface.

The curing time in the press will probably have effect on the strength of the glued joints; for example, one specimen kept in the press for a small length of time might give poorer or equal result than one kept for a longer period of time. In case of a poorer result using a small curing time, longer curing time will be desirable to obtain better joints; on the other hand, if equal results are obtained for different curing time, smaller curing time will be desirable from economic standpoint.

The effect of ageing of the final glued joints is an important factor particularly to the prospects of commercialization. Fortunately however, it has been observed that ply-wood veener samples glued with G-D adhesive and almost a year old, have not shown any deterioration even after occasional exposure to snow and boiling water.

Theory

In order to study these four variables we adopt the following statistical procedure:

1. Let these four variables be factors F_1,F_2,F_3, and F_4.

2. Each of these factors are to be estimated at 5 levels.

3. By use of 5X5 Graeco-Latin square each level of the factor F_1 is attempted in conjunction with all levels of the other three factors; thus the assessment of the effect of factor F_1 is not biased by any possible effect(s) of the other factor(s).

	I	II	III	IV	V
1	A,α	B,β	C,γ	D,δ	E,ϵ
2	E,δ	A,ϵ	B,α	C,β	C,γ
3	D,β	E,γ	A,δ	B,ϵ	C,α
4	C,ϵ	D,α	E,β	A,γ	B,δ
5	B,γ	C,δ	D,ϵ	E,α	A,β

In the above arrangement of the Graeco-Latin square, the notations have the following significance:

1, etc. : Proportion of solid: solvent ratio

I, etc. : Mixing times, minutes

A, etc. : Curing time in the press, hours

α, etc. : Age of the glued joints, days.

(4) In a fully orthogonal 5 x 5 square, six alphabets are required. In our experimental setup we have used four alphabets in order to keep room for error terms. However, the remaining two alphabets are utilized to cancel out any possible change in the "master-batch" during the preparation of the "specimens".

(The "master-batch" would be the several batches of the Run #139A, stored to carry out all the experiments. The "specimens" are $(A,\alpha)^{I}_{1}$, $(D,\varepsilon)^{III}_{5}$ etc.). Any time trend in the "master-batch", would be detected by way of selected replications of 12 specimens, made at different time with reference to the time of preparation of the "master-batch".

Now the exact design of the experiment could be obtained by choosing some values (from our previous experience) for all different levels of all these factors.

1, 2, 3, 4, 5: 1, 2, 3, 4, 5 gms of alcohol-dried product in 20 ml of a 5% $Ca(OH)_2$ slurry in distilled water.

I, II, III, IV, V: 5, 10, 15, 20, 25 minutes.

A, B, C, D, E: 1.5, 3, 6, 12, 24 hours.

α, β, γ, δ, ε: 0, 4, 16, 36, 64 days.

Substituting these values, we obtain:

$$^{1}(1.5,0)^{I}_{1} \quad ^{7,30}(3,4)^{II}_{1} \quad ^{13}(6,16)^{III}_{1} \quad ^{19,33}(12,36)^{IV}_{1} \quad ^{25}(24,64)^{V}_{1}$$

$$^{12,37}(24,36)^{I}_{2} \quad ^{18}(1.5,64)^{II}_{2} \quad ^{24,35}(3,0)^{III}_{2} \quad ^{5}(6,4)^{IV}_{2} \quad ^{6,29}(12,16)^{V}_{2}$$

$^{23}(12,4)^{I}_{3}$ $^{4,27}(24,16)^{II}_{3}$ $^{10}(1.5,36)^{III}_{3}$ $^{11,36}(3,64)^{IV}_{3}$ $^{17}(6,0)^{V}_{3}$

$^{9,32}(6,64)^{I}_{4}$ $^{15}(12,0)^{II}_{4}$ $^{16,28}(24,4)^{III}_{4}$ $^{22}(1.5,16)^{IV}_{4}$ $^{3,26}(3,36)^{V}_{4}$

$^{20}(3,16)^{I}_{5}$ $^{21,34}(6,36)^{II}_{5}$ $^{2}(12,64)^{III}_{5}$ $^{8,31}(24,0)^{IV}_{5}$ $^{14}(1.5,4)^{V}_{5}$

This compact design has all the details of the specimens tagged with the symbols. Thus

$^{1}(1.5,0)^{I}_{1}$ = 1 gm. of alcohol dried sample (of run #139A) is mixed with 20 ml of 5% $Ca(OH)_2$ slurry for 5 minutes, cured for 1.5 hours in the press and tested without ageing the glued joints. Symbol 1 on the lefthand corner indicates that this is the first experiment.

$^{21,34}(6,36)^{II}_{5}$ = 5 gms of alcohol dried sample (of run #139A) is mixed with 20 ml of 5% $Ca(OH)_2$ slurry for 10 minutes, cured for 6 hours in the press and tested after ageing for 36 days. This is a replicate run - constituting the 21st and 34th experiments.

Results

The tensile strength values (in p.s.i.) as obtained for 37 experiments are tabulated in Table 12. All of these values are the means of two experiments each. The idea was to get more consistent results. These data are coded by subtracting 592.75 and the ANOVA is computed for the regular 5 x 5 orthogonal graeco-latin square design (vide Table 13). From

Table 12. Tensile Strength Data of Graeco-Latin Square Design

				5 x 5 Square	Replications		
$^{1}122.50$	$^{7}732.85$	$^{13}599.40$	$^{19}461.50$	$^{25}759.00$	$^{30}557.8$	$^{33}599.35$	
$^{12}636.55$	$^{18}500.95$	$^{24}715.35$	$^{5}595.00$	$^{6}719.75$	$^{37}818.15$	$^{35}403.3$	$^{29}752.45$
$^{23}730.60$	$^{4}800.65$	$^{10}787.50$	$^{11}750.30$	$^{17}533.75$	$^{27}875.00$	$^{36}945.05$	
$^{9}966.85$	$^{15}890.5$	$^{16}665.00$	$^{22}770.05$	$^{3}859.65$	$^{32}815.95$	$^{28}791.85$	$^{26}846.55$
$^{20}815.95$	$^{21}877.20$	$^{2}796.25$	$^{8}840.00$	$^{14}767.75$	$^{34}759.10$	$^{31}723.15$	

Table 13. ANOVA Table for 5 x 5 Square using Regular Data. (Data of Table 12 were coded by subtracting 592.75 and used here)

Source	d.f.	SS	MS
Solid:Solvent Ratio (1, etc.)	4	315,086.84	78,771.71
Mixing Time (I,etc.)	4	33,199.70	8,299.92
Curing Time (A,etc.)	4	98,330.07	24,582.51
Ageing Time (α, etc.)	4	56,572.84	14,143.21
Error	8	221,968.22	27,746.02
Total	24	725,157.67	153,543.37

this ANOVA Table 13, it is obvious that, solid:solvent ratio plays the major role; curing time might act as an important factor but others are just noise.

The effect of any possible time trend is evaluated by calculating the residuals in the following way. The mean value of every row was subtracted from every single value in the same row. Thus these residuals (Table 14) have only the effect of solid:solvent ratio incorporated in them. A plot (Figure 8) of these residuals against time does not indicate any time trend. This led to the idea of computing the legitimate replication error estimate which is worth 12 degrees of freedom. This was done by computing the mean square of the differences obtained in the tensile strength values of those cases where we had replications. Remarkable is the fact that curing time becomes little bit more significant by considering $\frac{\text{Curing time MS}}{\text{Replication MS}}$, rather than $\frac{\text{Curing time MS}}{\text{Error MS}}$. To reason out two questions (1) Why error mean square (EMS) is greater than replication mean square (RMS)? and (2) Why Experiment # 1 yielded the havoc making result, which is very very low compared to other results?, one would very legitimately suspect a possible interaction between the factors solid:solvent ratio and curing time. So the interaction mean square (IMS) is computed (Table 15) and is found to be 19,468.10 which, indeed, is very significant. Almost obvious conclusion is to blame the experiment #1, which is throwing the lions share in the interaction situation. This is confirmed by computing a missing value estimate for a regular latin square (disregarding the alphabets for ageing) so as to linearize the problem and

Table 14. Residuals of the Coded Data as Obtained from Table 12

5 x 5 Square						Replications	
-424.99	185.36	51.91	-85.99	211.51	10.31	51.86	--
-6.13	-141.73	72.67	-47.68	77.07	175.47	-239.38	109.77
-44.09	25.96	12.81	-24.39	-240.94	100.31	170.36	--
141.05	64.70	-160.80	-55.75	33.85	-9.85	-33.95	20.75
18.90	80.15	-1.25	42.95	-29.30	-37.95	-70.90	--

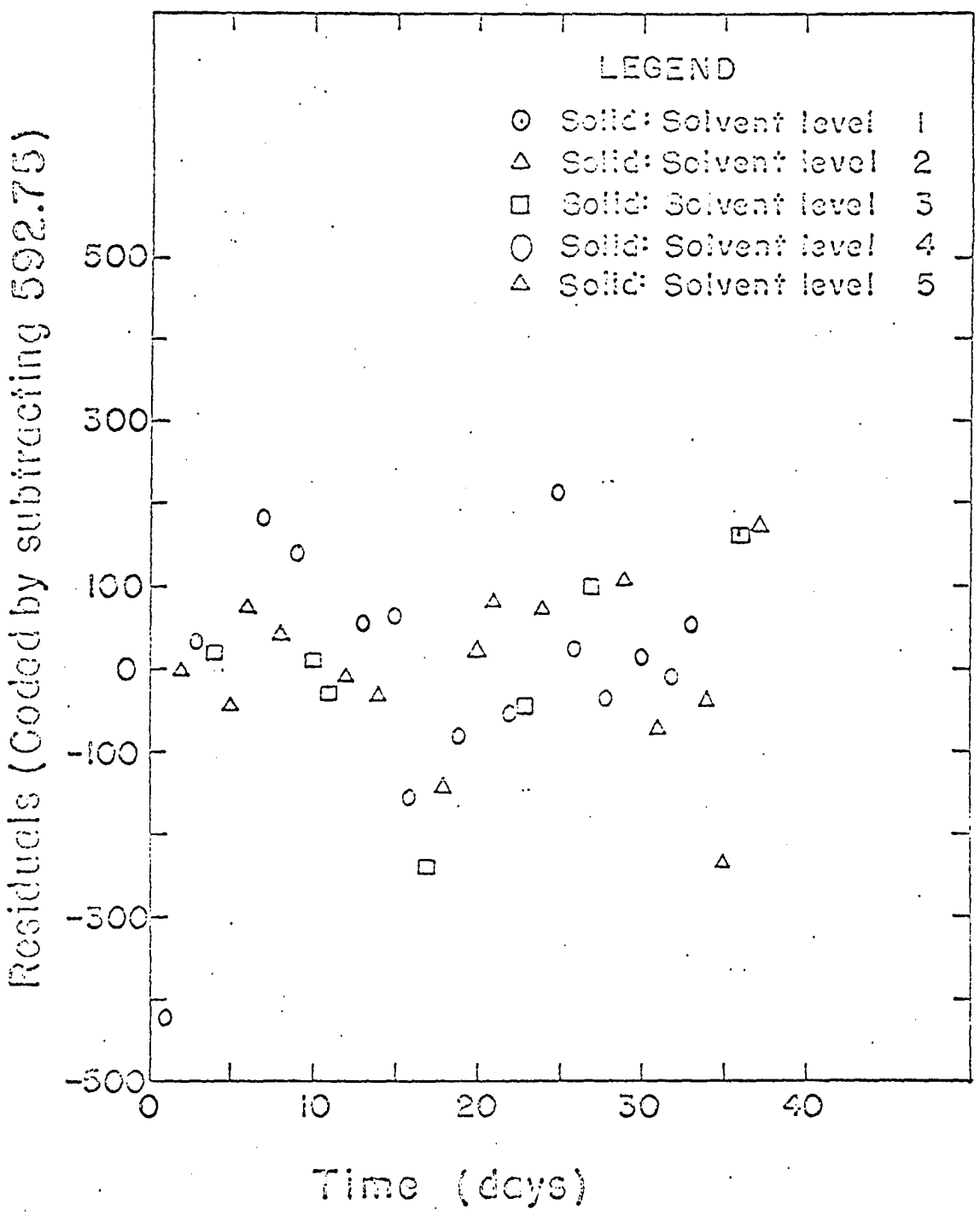


Fig. 8 Plot of residuals (taken from table 14) vs. time

Table 15. Calculation of Interaction Mean Square for the Factors Solid:Solvent Ratio vs Curing Time (Data of Table 12 were coded by subtracting 592.75 and used here)

Interaction sum square = $L^2 = \sum\sum_{ij} (x_{ij} - \bar{x}_{i\cdot} - \bar{x}_{\cdot j} + \bar{x}_{\cdot\cdot})^2$

where i = 1, 2, 3, 4, 5(Solid:Solvent)
j = A, B, C, D, E(Curing time)

$\bar{x}_{1\cdot} = -57.70$ $\quad \bar{x}_{\cdot A} = -3.00$
$\bar{x}_{2\cdot} = 40.77$ $\quad \bar{x}_{\cdot B} = 182.07$
$\bar{x}_{3\cdot} = 127.81$ $\quad \bar{x}_{\cdot C} = 121.69$ $\quad \bar{x}_{\cdot\cdot} = 115.04$
$\bar{x}_{4\cdot} = 237.66$ $\quad \bar{x}_{\cdot D} = 126.97$
$\bar{x}_{5\cdot} = 226.68$ $\quad \bar{x}_{\cdot E} = 147.49$

x_{ij}	L	x_{ij}	L	x_{ij}	L
-470.25	-294.71	297.75	48.16	2.25	-45.17
43.80	-29.42	284.45	51.12	157.55	-37.29
137.85	-1.89	6.65	57.70	177.30	57.68
374.10	129.79	122.60	14.80	247.25	-11.88
223.20	-70.51	194.75	183.98	166.25	191.50
140.10	130.77	72.25	-197.86	127.00	74.30
-91.80	-14.53	203.50	35.11	-59.00	-193.46
207.90	47.64	-131.25	- 85.48	266.90	-37.79
				175.00	66.36

$\sum L^2 = 311{,}489.64$

Interaction Mean Square = $\frac{\sum L^2}{df} = \frac{\sum L^2}{16} = 19{,}468.10$

then recomputing the latin square ANOVA (Table 16). We note that the EMS using missing value estimate is almost close to RMS computed before.

Not completely explained are the very low mean square values for the other factors.

The graphical analog of the ANOVA (5 x 5) of both regular and missing data is given in Figure 9. The behavior of solid:solvent ratio in either cases led us to suspect an explosion type characteristics. This called for some additional experiments, in which the solid: solvent ratio was varied. Other conditions were picked up from Figure 9, as the optimized conditions : viz, 10 minutes of mixing, 3 hours of curing and no ageing of the glued joints. When these additional experiments were carried out, Figure 10 was obtained. The tensile strength values for levels 1, 2, 3, 4 and 5 of solid: solvent ratio were derived by averaging the individual rows of 5 x 5 latin square. Also plotted in the same graph (Figure 10) are the values obtained by including the additional 12 replicate runs and averaging the rows over 7, 8, 7, 8 and 7 individual results (of Table 12).

Thus the optimized conditions are solid:solvent ratio-4·25gms/20 ml solvent.

Mixing time	10 minutes
Curing time	3 hours
Ageing time	0 days

Table 16. ANOVA Table for 5 x 5 Square using a Missing Value Estimate for the Observation in Experiment # 1 (Data of Table 12 were coded by subtracting 592.75 and used here)

Missing observation in an m x m latin square

$$M = \frac{m\,(R + C + T) - 2S}{(m-1)\,(m-2)}$$

where

m = 5
R = Row sum = 181.75
C = Column sum = 778.95
T = Treatment sum = 455.25
S = Sum of actual observation = 3346.35

whence

M = 32.26

Source	df	SS	MS
Solid:Solvent ratio (1, etc.)	4	181,878.27	45,469.56
Mixing time (I, etc.)	4	20,030.72	5,007.68
Curing time (A, etc.)	4	20,096.09	5,024.02
Ageing time (α, etc.)	$(4)(\frac{11}{12})$	9,162.82	2,290.70
Error	$(8)(\frac{11}{12})$	148,171.25	18,521.40

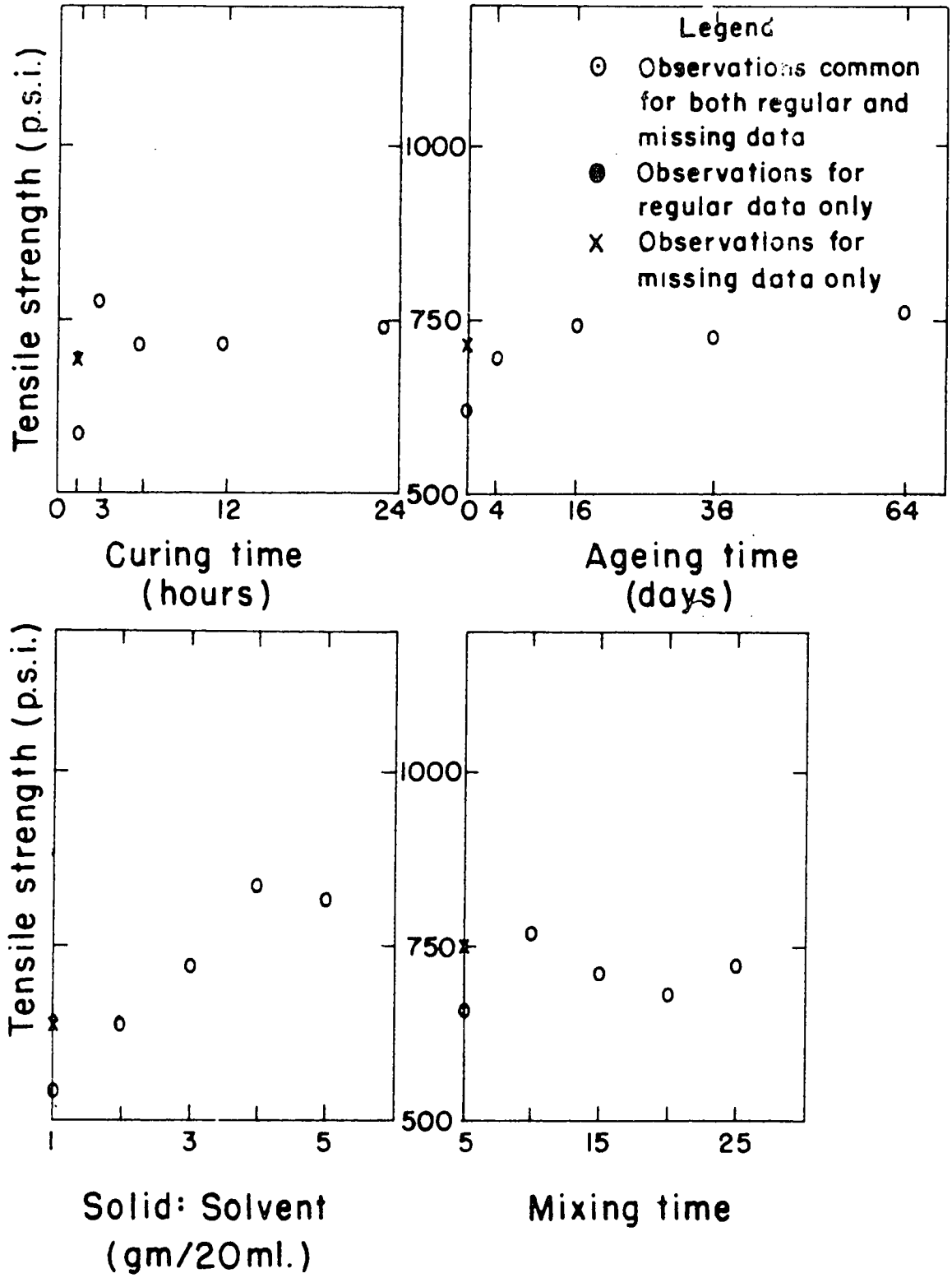


Fig. 9 Graphical analog anova tables 13 & 16

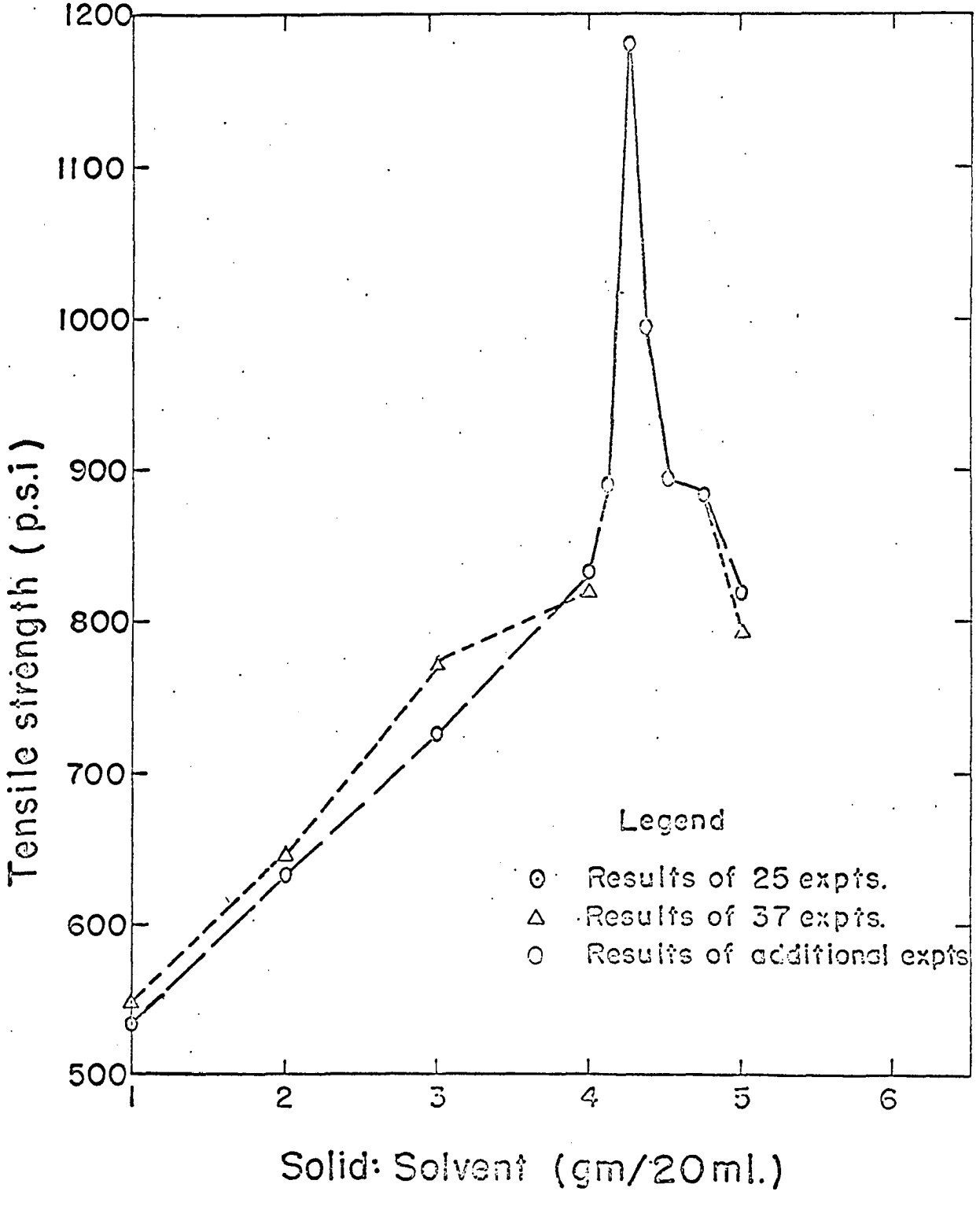


Fig. 10 Plot of tensile strength (p.s.i.) vs. Solid: Solvent ratios

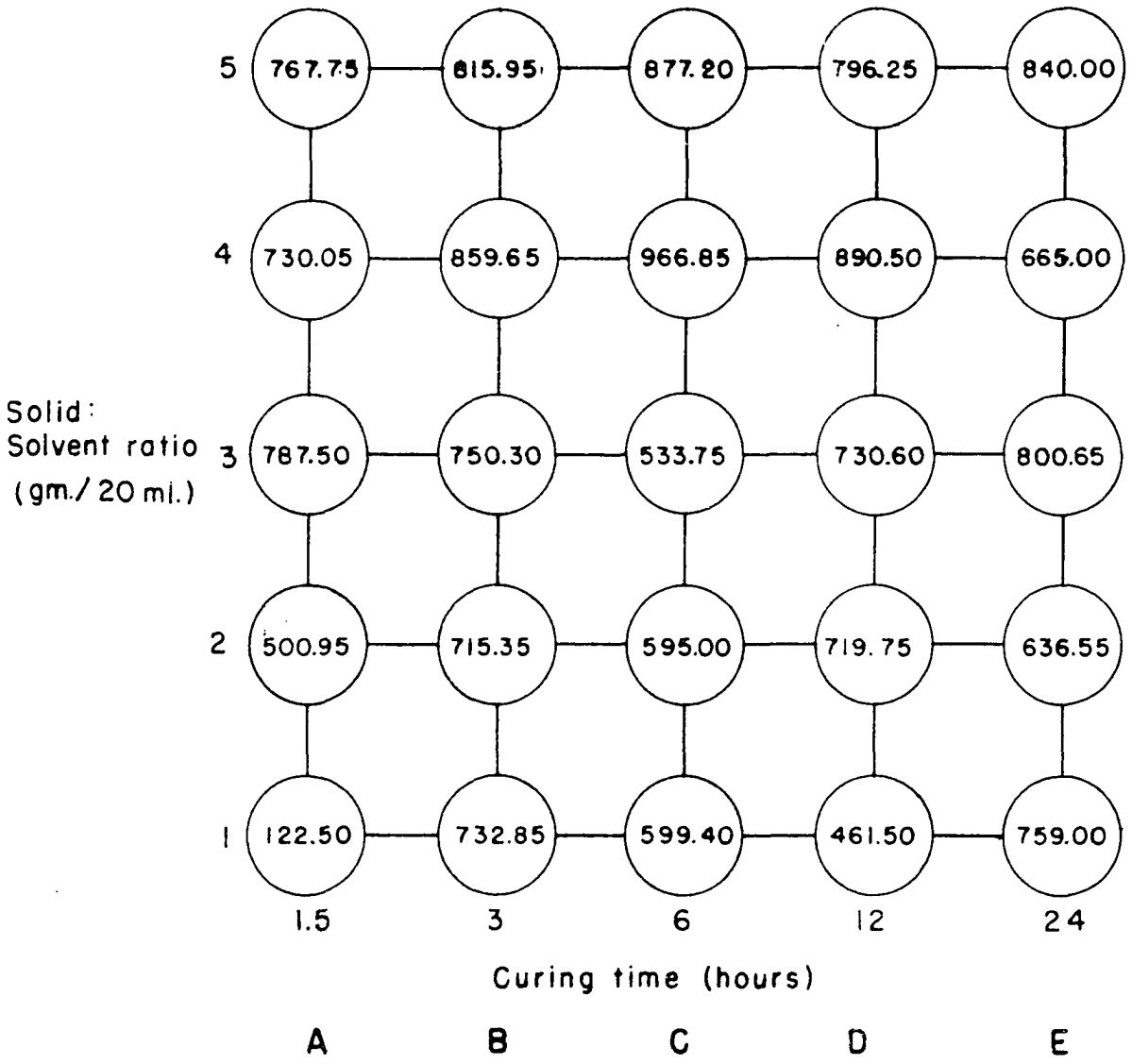
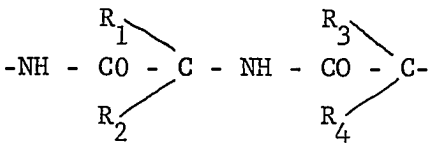


Fig. II Block diagram showing the interaction of solid : solvent vs. curing time

Part III. Fundamentals

In this part of the experimental results, the aim was to secure some fundamental knowledge about the reaction of dialdehyde starches and the proteins of gluten. Since gluten represents an exceedingly complex protein system, the emphasis was given to DAS. The proteins of gluten are represented by the general polypeptide structure



for convenience. The structure of DAS is known. So, in order to gind the extent of cross-linking, the residual aldehyde groups of the DAS were estimated. Degradation experiments were carried out under controlled conditions and products of degradation were isolated and identified to locate which of the two aldehyde groups of the glucose residues of DAS is more reactive towards a protein system.

Glutenin and gliadin fractions of gluten were isolated and reacted separately with DAS. The performic acid degradation product of glutenin was also made by use of the method of Nielsen (11) ; this was reacted with DAS. Various reaction products of parent gluten and its components with DAS were subjected to micro estimations of C, H, N and S to get any relevant conclusion.

The work done in this chapter can be arranged in the following fashion:

Aldehyde Estimation

Aldehyde units of DAS and G-D product were estimated by using two well known methods. These are (1) rapid estimation of dialdehyde content of periodate oxystarch through quantitative alkali consumption Hofreiter, Alexander and Wolff (24) and (2) determination of dialdehyde units in periodate oxidized cornstarches by the borohydride method of Rankin and Mehltretter (25).

In the method of Hofreiter *et al.*, known amount of the sample (about 0.1500 to 0.2000 gm) of known moisture content is weighed into an Erlenmeyer flask. About 10 ml of standardized, carbonate-free 0.25N sodium hydroxide is added, via pipet, and the flask is gently swirled and placed on a steam bath for 1 minute. There should be very rapid flow of steam. The flask is then cooled immediately under running tap water and 15 ml of standardized 0.25N sulfuric acid is added by pipet. The contents are diluted to 60 ml by adding water and 1 ml of 0.2% phenolphthalein indicator. Titration of the acid solution is carried out using 0.25N sodium hydroxide, delivered from a 10 - ml microburet.

The theory in this method is that, when polyaldehydes produced by the periodate oxidation of carbohydrates, are treated with alkali, internal Cannizzaro reaction occurs. The percentage of dialdehyde units in oxystarch is thus given by the equation

$$\frac{\text{Total meq. base - Total meq. acid}}{\text{dry sample wt., mg.}} \times \frac{100}{161} = \%$$

dicarbonyl units.

In this method of Rankin and Mehltretter, about 0.1000 gm of the sample is weighed into a 50 ml reaction flask. A stirring magnet is added, followed by 4.0 ml of 0.1M boric acid. The apparatus is assembled as shown in Figure 12. The leveling bulb is filled with 300 ml of water saturated with hydrogen. Air is expelled from the system by moving the leveling bulb to the top of the buret with the three way stopcock open to the atmosphere. The stopcock is closed when the buret is completely filled with water. The leveling bulb is then lowered and the stopcock opened to the reaction flask. This operation is repeated until water fills the capillary portion of the buret with the three way stopcock open to the reaction chamber. Three milliliters of 0.26 M sodium borohydride solution is then pipetted into the dropping funnel. The borohydride solution is added to the sample and boric acid mixture and the stopcock closed when the solution level reaches the capillary portion of the funnel. All the borohydride is washed into the reaction flask with 3 ml (pipet) of water by the same procedure. The addition of a constant volume of solution is assured by maintaining the same level of liquid in the capillary throughout the reaction. Reduction of the aldehyde groups is complete within 2 hours. The mixture is then acidified by slow addition of 3 ml of 2N sulfuric acid to liberate hydrogen from unreacted sodium borohydride. This addition is made as above, leaving the capillary tube filled with acid. The reaction mixture then stirred for 5 minutes to dissipate hydrogen bubbles in the solution. Buret readings are recorded at the end of 15 and 30 minutes by aligning the water meniscus of the

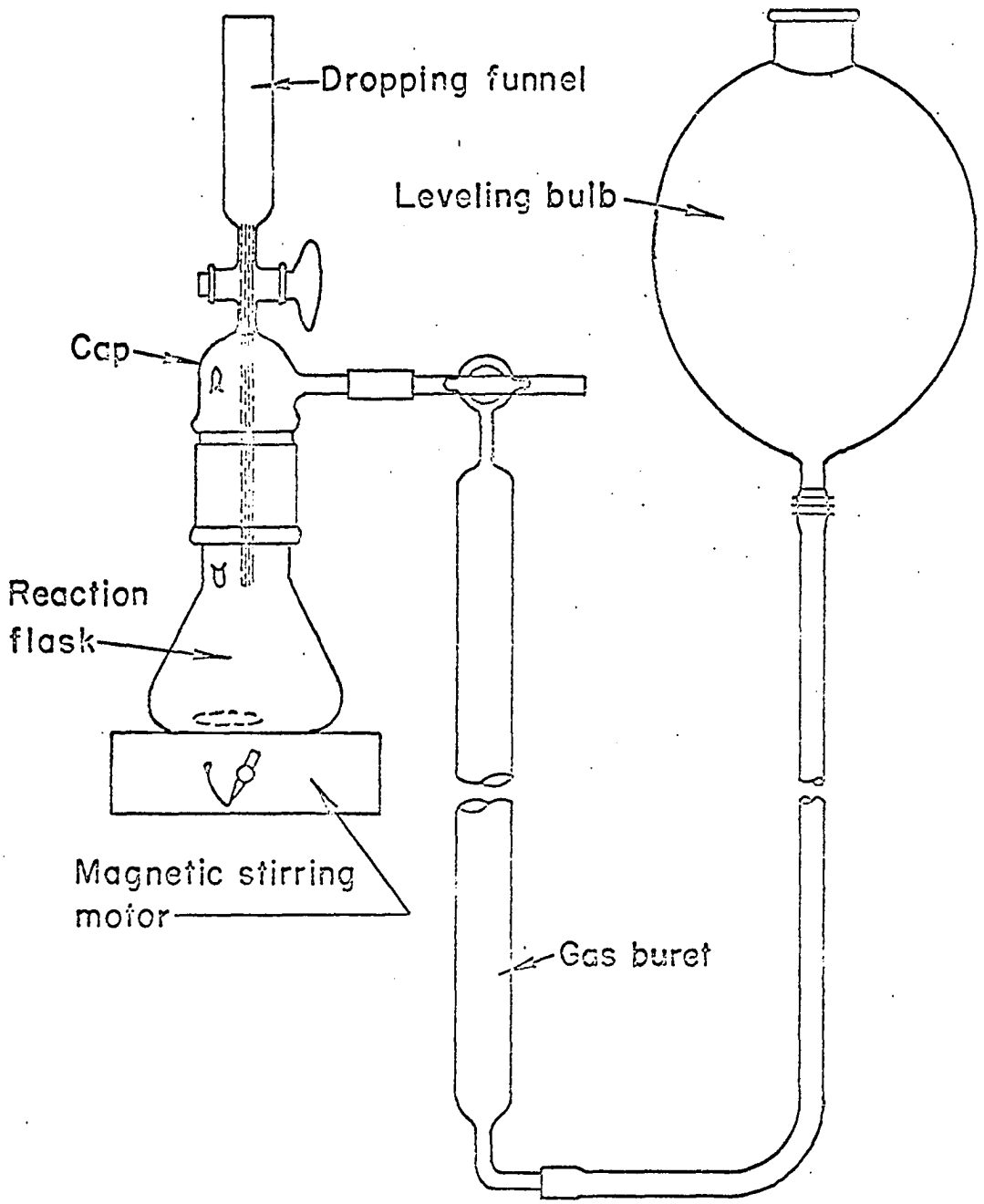


Figure 12 Apparatus for sodium borohydride method

leveling bulb with that of the buret. The readings are averaged.

The percentage dialdehyde units in oxystarch is given by

$$\% \text{ dialdehyde units} = \frac{(\text{ml. } H_2 \text{ from blank} - \text{ml. } H_2 \text{ from sample}}{\frac{(\text{dry sample wt. (gm)} \times 2 \times 22{,}400)}{160}}$$

The volume of hydrogen involved during sample and blank runs must be corrected for vapor pressure of water at 25°C., and to standard conditions of temperature and pressure.

Results are shown in Table 17. Percentage dialdehyde units is calculated only in the case of DAS; it can not be done in other cases because the polymer unit is not known for gluten and G-D product.

Degradation Studies of the G-D Product

Very often degradation study has been a very powerful tool in understanding the high polymeric reactions. Dialdehyde starches on drastic hydrolysis with aqueous acid gives substantial yields of glyoxal and D - erythrose by scission of the acetal bridge linking the first two to the other four carbon atoms of the original oxidized glucose residue (14). If the carbonyl group on C_3 remains free on the G-D product, then on reduction with sodium borohydride and subsequent acid hydrolysis, erythritol will be obtained from the reaction product. Similarly, if the aldehyde group on C_2 remains free, on reduction and hydrolysis, glycolaldehyde will be obtained (16).

Degradation of DAS with dilute mineral acid leads to varied changes in the molecule and the recondensation of hydrolytic products is also

Table 17. Estimation of Dialdehyde Units

Rapid estimation method

Standard NaOH 0.2660N Standard H_2SO_4 0.2403N

Sample	NaOH from microburet ml	% dialdehyde units
DAS (0.1572 gm)	6.59	93.1
DAS (0.1685 gm)	6.67	94.0
GD product (0.1515 gm)	4.01	
GD product (0.1660 gm)	4.07	

Borohydride reduction method

Standard boric acid 0.1010M Standard borohydride 0.2644M

Sample	Difference in Volume of H_2 collected, ml	% dialdehyde units
DAS (0.1001 gm)	24.8	99.4
DAS (0.0110 gm)	24.6	98.6
GD product (0.1004 gm)	7.8	
GD product (0.1001 gm)	7.2	
Gluten (0.1003 gm)	0.7	
Gluten (0.1004 gm)	0.5	

not unlikely. The method used for degradation was that of Grangaard, Gladding and Purves (27). In this method, the action of 10% methanolic hydrogen chloride on periodate oxystarch produces about half the expected amount of glyoxal tetramethyl acetal, which can be isolated by distillation.

The quantitative determination of the glyoxal units present in G-D product before and after reduction with sodium borohydride is used as a means for determining the participation of this unit in the reaction with proteins. Glyoxal can be quantitatively determined by taking advantage of its reaction with 2, 4 di-nitro phenylhydrazine to form crystalline derivative of identifiable characteristics.

The estimation of dialdehyde cleavage can be carried out in the following way. The sample 0.1 to 0.2 gm of the oxystarch (or G-D product) is boiled under reflux for 20 hours with 25 ml of methanol containing 2.5 gms of hydrochloric acid. After cooling, the solution is made alkaline with 20 ml of nearly saturated sodium methylate (made by slow addition of metallic sodium to anlydrous methanol) in methanol and the volume is adjusted with methanol to 60 ml. The alkaline solution is quantitatively transferred to a 500 ml distilling flask with exactly 300 ml of an aqueous solution containing 6.5 gms of sodium chloride and 1 gm of sodium hydroxide. A water condenser is used in the atmospheric pressure distillation and the first, second and third 100 ml distillates are collected separately. Hundred milliliters of water is then added in one portion to the still and a fourth 100 ml of distillate is collected. A 50 ml aliquot of each of the four distillates is diluted with an equal volume of 2N hydrochloric acid and

hydrolyzed by heating for one hour on steam bath. The hydrolyzates are examined by 2,4 dinitrophenylhydrazine Fifty ml of nearly saturated solution of 2, 4 dinitrophenylhydrazine (in 2N hydrochloric acid) is added to 50 ml of each hydrolyzed fraction. The precipitates are filtered in sintered bed crucible, washed with 2N HCl and dried in a vacuum drier at 60^{o} for 12 hours and weighed. The amount of samples used and derivatives obtained by this method are reported in Table 18. These results are very significant as will be interpreted in the "Discussion" of this thesis. Just a glance to these data shows that quite a considerable amount of glyoxal is present in the degradation product obtained from the borohydride reduced G-D product and the magnitude is of the same order as observed in the cases of unreduced G-D product.

The borohydride reduction was carried out in anhydrous methanol as solvent. One tenth of 1-2 gm of G-D product was suspended in 6 ml of anhydrous methanol and sodium borohydride (0.012 gm in 1 ml anhydrous methanol) was added with vigorous stirring. Twenty five milligrams of sodium methylate was then added and the alkaline reaction reaction mixture was left overnight. An additional quantity of sodium borohydride (0.005 gm) was added and the reaction mixture was allowed to stand for another 6 hours. A minute crystal of methyl orange was added and the mixture was neutralized with dilute hydrochloric acid (diluted with anhydrous methanol). Fifteen milliliters of absolute methanol and 1.5 ml of concentrated hydrochloric acid were then added and refluxed to carry out the methanolysis.

Table 18. Estimation of the Degradation Product Obtained from G-D Product (both before and after reduction with borohydride)

	Wt. of original sample (gm)	Wts. of 2,4 - dimitrophenylhydrazine derivative			
		1st fraction	2nd fraction	3rd fraction	4th fraction
Before Reduction	0.1591	0.0050	0.0018	0.0014	0.0010
	0.1491	0.0056	0.0028	0.0018	0.0008
After Reduction	0.1817	0.0056	0.0026	0.0022	0.0008
	0.1947	0.0050	0.0026	0.0016	0.0012

Preparation of Some Special Products

The special products which were made include glutenin - DAS, gliadin - DAS and performic acid - treated - glutenin - DAS reaction products.

Preparation of glutenin and gliadin

The reactants glutenin and gliadin were prepared from the parent gluten by making use of the procedure used by Nielsen, Babcock and Senti (11). For our convenience we had somewhat scaled up their method. Nine grams of whole gluten was dissolved in 2700 ml of 0.1N acetic acid and 300 ml of 0.5 N sodium acetate was slowly added (pH 4.5) which resulted in the precipitation of the crude glutenin. Gliadin was then precipitated by making the solution molar with sodium chloride and by adjusting the pH to 7.0. The gliadin precipitate was then despersed in 0.01N acetic acid and lyophilized and stored in sample jar. A little over 1.5 gms of gliadin was obtained. Crude glutenin was purified by dispersing in 2700 ml of 0.1N acetic acid with the aid of a high-speed blendor (Waring) and 300 ml of 0.5N sodium acetate was slowly added to precipitate purified glutenin. The precipitate was dispersed in 0.01N acetic acid and lyophilized and stored in sample jar. About 3.5 gms of glutenin were obtained. Lyophilization method is described in Appendix.

Oxydation of glutenin with performic acid

The performic acid - degraded glutenin, which, according to Nielsen et al. (11), is the building unit of the proteins of wheat, was

prepared according to the method used by Hirs (26). Here again we had scaled up their original method for our own facilitation. Performic acid was prepared by adding 1.0 ml of 30% H_2O_2 (reagent, Mallinckdrot) to 20 ml of 89% formic acid (Matheson, Coleman and Bell, Inc.). The resulting solution was allowed to stand at room temperature (27^{o}) for 2 hours in a stoppered flask. In another flask 400 mg of glutenin (lyophilized) were dissolved completely in 15 ml of 89% formic acid, after which 2 ml of anhydrous methanol was added with stirring. The methanol was added to prevent freezing of the solution during the oxidation at -10^{o}. Occasional stirring was done magnetically and during stirring, the flask was taken out of the bath (ice-sodium chloride) which was maintained at -10^{o} to -15^{o}. The reactants were cooled for 20 minutes in the ice-salt bath and then mixed by pouring the acid into the flask containing glutenin. The reaction was allowed to take place at the same temperature for 3 hours. The contents of the flask were rinsed with 100 ml of ice water into a flask containing 650 ml of water at 0^{o}. The diluted solution was divided into three portions and taken in 1000 ml flasks. Each of the three portions were frozen immediately and lyophilized. The fluffy product obtained was stored in sample jar and kept.

Reaction of glutenin and 93% DAS

Glutenin was made in several batches. Seven and a half gms of glutenin were dissolved in 100 ml of 5% acetic acid. A DAS solution

was made in the same fashion as was done in Run #139A of Part I. The glutenin solution was then reacted with 15 ml of DAS solution at 26°C for 10 minutes. Gummy precipitate was obtained, which was very similar to the gluten - DAS reaction product. The precipitate was separated from the supernatant liquid by centrifugation and washed with distilled water. Part of it was alcohol washed and the rest of it was lyophilized. Thus we obtained two kinds of dried glutenin - DAS reaction products. As would be expected, the alcohol dried product was little bit coarse in feel wheras the lyophilized material was real fluffy.

Reaction of gliadin and 93% DAS

DAS solution in 5% $NaHSO_3$ was added dropwise to a dilute solution (1 gm per 100 ml) of gliadin in 5% acetic acid till the hydrophobic precipitate of gliadin - DAS reaction product separated from the bulk of the solution. After 10 minutes of reaction time the granular precipitate was centrifuged out, washed with distilled water and one half of it was alcohol washed and the other half was lyophilized.

Reaction of oxidized glutenin and 93% DAS

The product obtained by oxidizing 400 mg of glutenin was taken in 50 ml of 5% acetic acid solution and a DAS solution (2 gms per 75 ml of 5% $NaHSO_3$) was added drop by drop till slimy precipitate appeared. When no further precipitation occurred (about 4.8 ml of DAS solution), the addition of DAS solution was discontinued and the reaction was allowed to continue for 10 minutes. The product was centrifuged out and washed with water. The gummy product was lyophilized to obtain filmy and fluffy white product.

Microestimations of different products

The results of microestimations of the elements C, H, N and S, carried out with different samples, are shown in Table 19. The symbols used for different samples are explained as follows:

Symbol	Sample it stands for
G	gluten (as received)
D	DAS (as received)
P (A.W.)	GD product (alcohol washed)
P (lyo.)	GD product (lyophilized)
G (lyo.)	lyophilized gluten from a 5% acetic acid solution
GBS (lyo.)	gluten solution in 5% acetic acid reacted with 5% $NaHSO_3$ solution (without any DAS)
gt-D (lyo.)	glutenin - DAS product (lyophilized)
d-gt-D (lyo.)	performic acid - oxidized glutenin - DAS product (lyophilized)
gd-D (lyo.)	gliadin - DAS product (lyophilized)

Table 19 shows that GBS (lyo.) does not have more sulfur than G (lyo.); this means that, during the reaction of gluten and DAS, the excess bisulfite present in the system, most probably, does not attack the protein system. Glutenin and performic acid - oxidized glutenin appear to be more reactive towards DAS than the parent gluten; this is obvious from the low nitrogen figures of gt-D (lyo.) and d-gt-D (lyo.). Not explained are the high values of nitrogen content in GBS (lyo.), P (lyo.) and gd-D (lyo.).

Table 19. Microestimations of Different Samples[a]

Sample	Element analyzed for			
	C	H	N	S
G	49.96	7.51	13.82	-
D	39.77	5.88	-	-
P (A.W.)	44.43	6.53	13.78	-
P (lyo.)	49.28	7.18	15.30	1.89
G (lyo.)	-	-	13.69	2.34
GBS (lyo.)	-	-	15.08	2.21
gt-D (lyo.)	-	-	9.98	2.88
d-gt-D (lyo.)	-	-	7.77	3.16
gd-D (lyo.)	-	-	15.02	2.89

[a]These samples were analyzed by Drs. Weiler & Strauss Microanalytical Laboratory, 164 Banbury Road, Oxford, England.

DISCUSSION

1. The present study on the reactions of dialdehyde starches and wheat proteins has resulted in an addition to the existing protein base adhesives. This adhesive, named as 'GD adhesive' is excellent for wood to wood bonding (Run #139A, Combination 7; p. 34). In fact, this adhesive can be classed as one of the rare cases where a protein base polymer can withstand water for several hours.

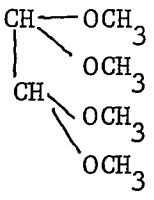
2. Statistical experiments carried out with this adhesive using completely randomized design model, has revealed that surface conditions are more important than all the process variables combined (p. 46). This leads us to an indirect proof, that, although the proteins of wheat gluten are pretty complex in nature, this new adhesive could be synthesized within proper tolerances by using the reaction conditions of Run #139A.

3. The conditions of adhesion using GD adhesive have been optimized by using a 5 x 5 fully orthogonal graeco-latin square design plus a random number of 12 replicas. The variables analyzed are solid:solvent ratio, mixing time, curing time and ageing of glued joints. The best solvent was chosen after trying 7 different kinds of probable solvents (p. 47). A 5% calcium hydroxide slurry in distilled water proved to be the best solvent for the gluten - DAS adhesive. Of all these four variables analyzed, the solid:solvent (solid = GD adhesive and solvent = 5% $Ca(OH)_2$ slurry) is most important. The facts that, the master-batch does not show any time trend (Figure 8, p. 59) and the strength of glued joints does not decrease with age (Figure 9, p. 63), are the most

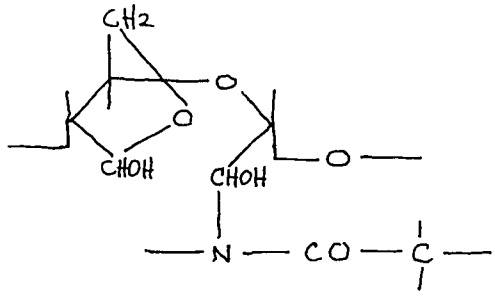
encouraging results that one may welcome with the idea of pilot plant study and eventual commercialization of the process. This set of statistical analyses has also pointed out the danger signal of the interaction between the factors solid:solvent and curing time at the 1 factor level combination (Experiment #1, Table 12, p. 55).

4. The free aldehyde contents of GD product is considerably lower than those of DAS. This is because of the fact that DAS is the minor component in gluten - DAS product and some aldehyde groups are cross-linked. Evidence of very low carbonyl content in pure gluten indicates that, in the cross-linked polymer DAS chain has quite a number' of residual aldehyde groups (Table 17; p. 70). This observation is in accordance with the early work of Sloan et al. (13), who reacted dialdehyde starches to urea and observed that one carbonyl group per repeating unit, remained free. The important thing to note is that the reactivity of the aldehyde groups plays a vital role in the reaction of DAS and a protein system. Although the proteins of wheat gluten represent a very complex system, DAS has shown a qualitative identity to its behavior toward urea.

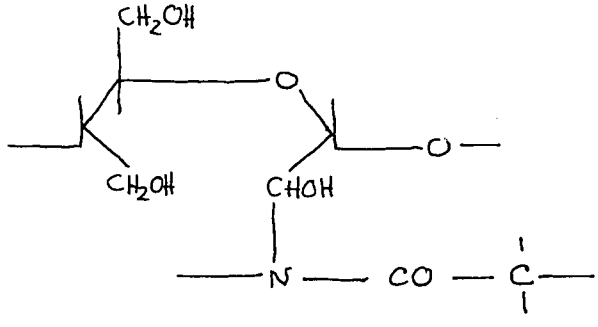
5. The degradation experiments have answered the obvious question--which carbon atom (C_2 or C_3) of each glucose unit of parent starch is more reactive? The formation of 2,4- dinitrophenylhydrazine derivative unambiguously indicates the presence of tetramethyl acetal of glyoxal (p.73)



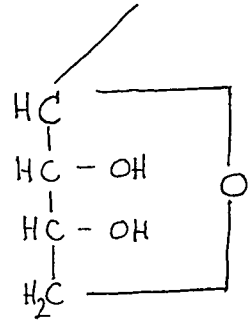
in the methanolysis-product of GD adhesive both before and after borohydride reduction. This can only result from a situation

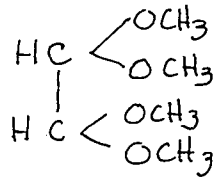


so that, during borohydride reduction the third carbon atom gets reduced



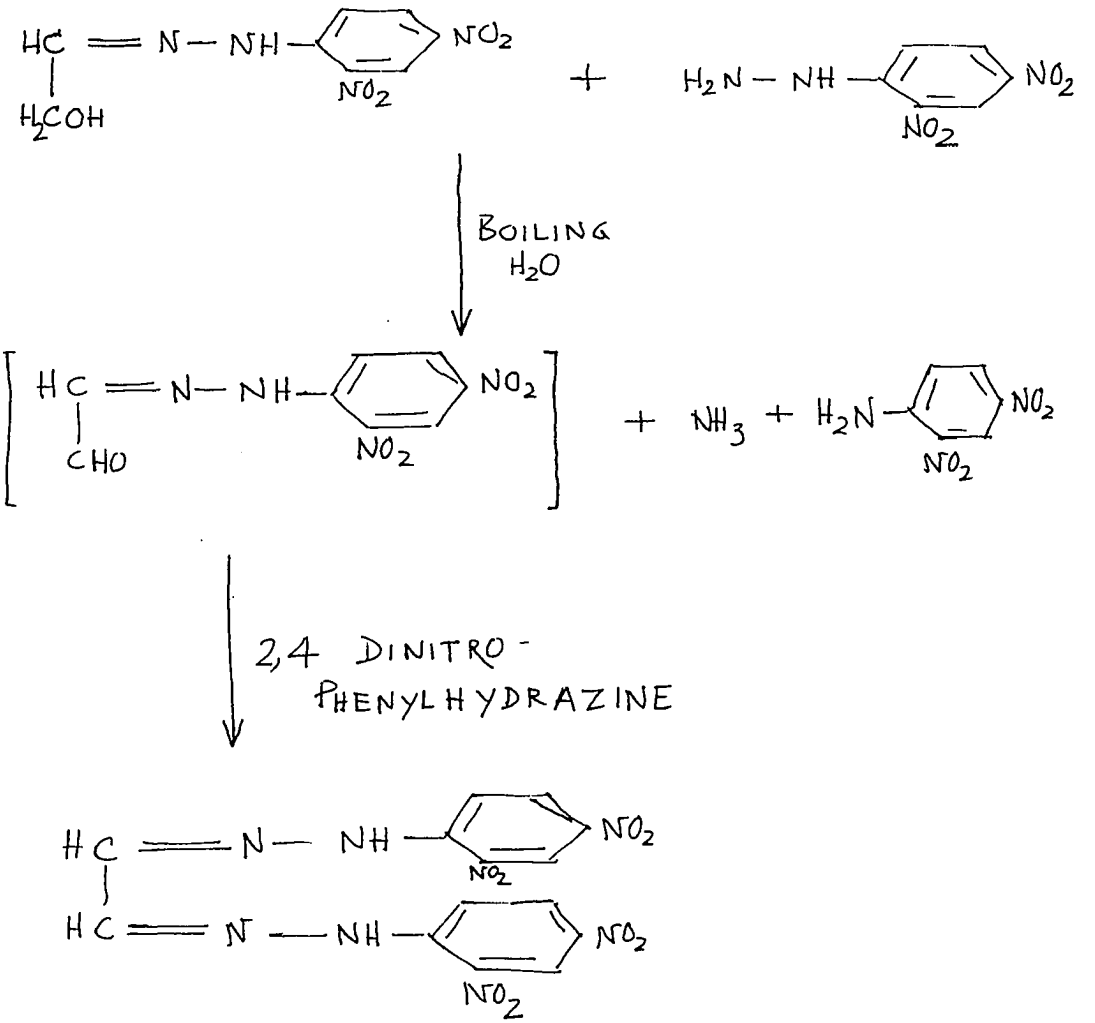
and upon subsequent methanalysis, one obtains methylerythroside and glyoxal tetramethyl acetal



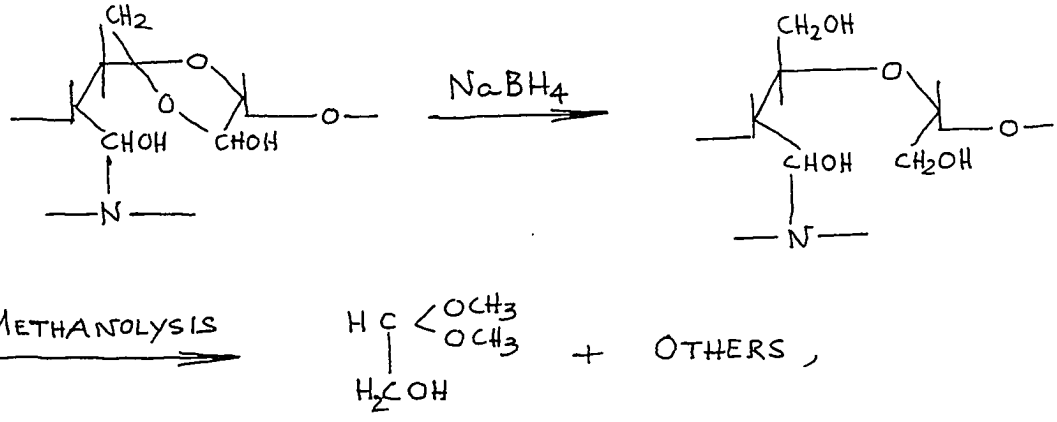


The possibility that 2,4- dinitrophenylhydrazine derivatives are derived from glycolaldehyde is ruled out by the observation of insolubility in water and alcohol.

The final confirmation that, 2,4- dinitrophenylhydrazine derivative of glyoxal did not result from the reaction



and that, glycolaldehyde did not come from the following sequence



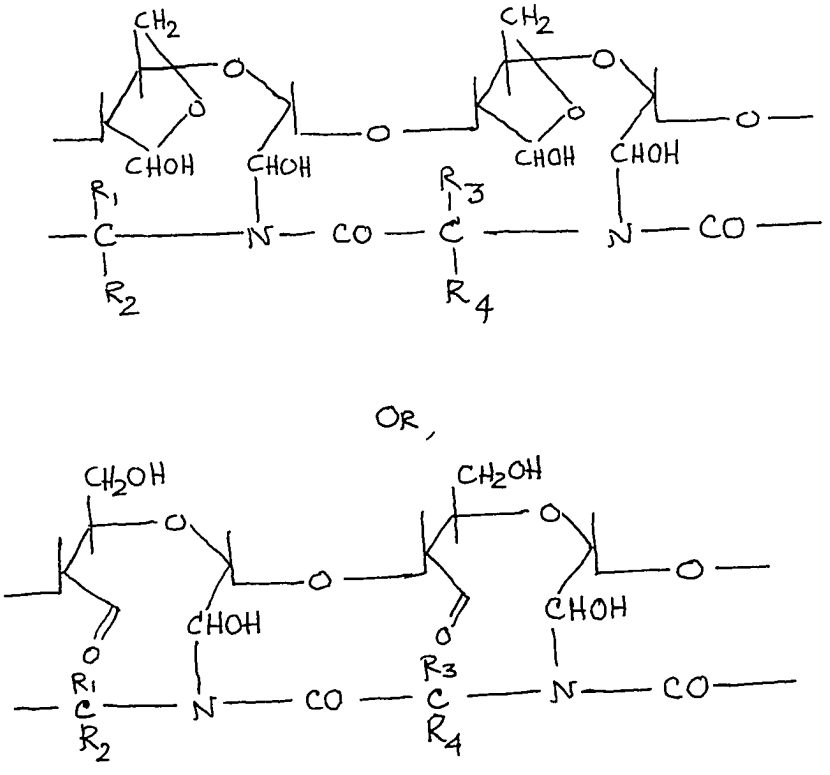
is obtained by carrying out precipitation at room temperature (only glyoxal does it), filtering the osazone of glyoxal and heating the filtrate when no further precipitation occurred. This was again checked with a blank run using 30% glyoxal solution, about 50% of which was reduced with sodium borohydride (on mole basis).

The results of degradation experiments apparently contradict the findings of Nayudamma, Joseph and Bose (16) who were working on the reactions of collagen and DAS. They had estimated the glyoxal, obtained from collagen treated DAS both before and after borohydride reduction, by using a colorimetric method (Ariyama) with Benedict's uric acid reagent. They found that the color of the reduced sample was of much lower intensity than that of the non-reduced sample. This was attributed to the presence of free carbonyl groups on C_2 in the DAS - tanned collagen.

The results obtained in our laboratory are exclusively quantitative in nature and hence, more sound than those obtained by using colorimetric

method. This would lead us to accept that the second carbon atom is more reactive than the third and hence the free carbonyl groups in GD product are mostly on C_3 of every repeating unit in DAS chain.

Thus we may represent the gluten - DAS product by the following structural formula:



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A. K. C.

APPENDIX A. SOLUBILITY OF DAS IN $NaHSO_3$

Preliminary studies of the solubility of DAS in $NaHSO_3$ solution have been made (Table A_1). Both 93% and 62% oxidation level DAS was used, the solvent being 5% $NaHSO_3$ in distilled water. Solution temperature was 68° - $72^{\circ}C$ and time required one hour. In both the cases, it was observed that density and viscosity of the solution increased with the concentration of DAS. The fact that pH increased in a regular manner (in the case of 93% DAS) is probably indicative of complex formation of the -CHO groups of DAS with $NaHSO_3$ at a particular temperature (2).

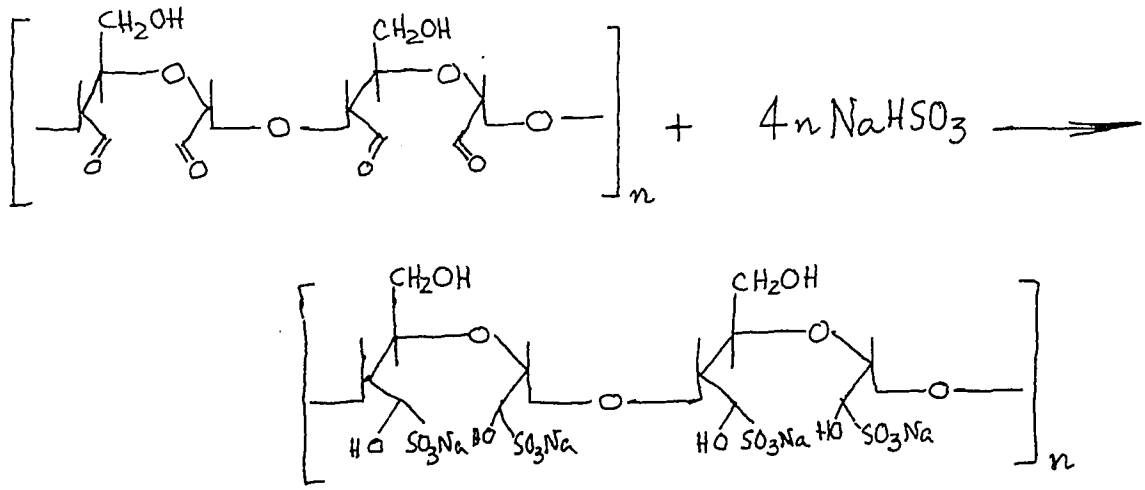

Table A_1. Solubility of DAS in 5% $NaHSO_3$ Solution

% DAS	Amount of DAS (gms)[a]	Density of the solution (gm/cc)	pH	Viscosity
93	1.1304	1.0319	3.9	3.8678
	1.9900	1.0302	4.1	15.1495
	3.0845	1.0382	4.0	15.5714
	3.9885	1.0421	4.2	too viscous
	5.0847	1.0658	4.6	too viscous
62	1.0076	1.464	4.7	1.7383
	2.1245	1.0306	4.85	1.7827
	3.0527	1.0378	4.6	too viscous
	4.2034	1.0477	4.4	too viscous
	5.0743	1.0646	4.6	too viscous

[a] In 60 ml of 5% $NaHSO_3$ solution in distilled water.

APPENDIX B. TENSILE STRENGTH TESTING METHOD

The tensile strength testing machine used to test the adhesive joints was an ASTM machine for testing briquettes. Two blocks of maple wood glued together, were of exact dimensions to fit the two sockets held in a vertical position. One of the two (which are identical) sockets (jaws) is shown in Figure A1. The glued joint was pulled vertically (so as to apply tension) by rotating a worm and screw gear which was geared to the bottom part of the lower jaw. The deflection (in the beam), as a result of tension was measured by shot flow method. Each pound of lead shot released to bring the beam back to its original position, was equivalent to 70 p.s.i. of tensile strength in the apparatus used. The complete machine is shown in Figure A2.

The general method used for testing was as follows. The G-D product (alcohol dried) was dispersed in the specific solvent and stirred very thoroughly till a homogeneous paste was obtained. A portion of this pasty mass was used to glue maple wood blocks which would fit the ASTM testing machine C77-37.

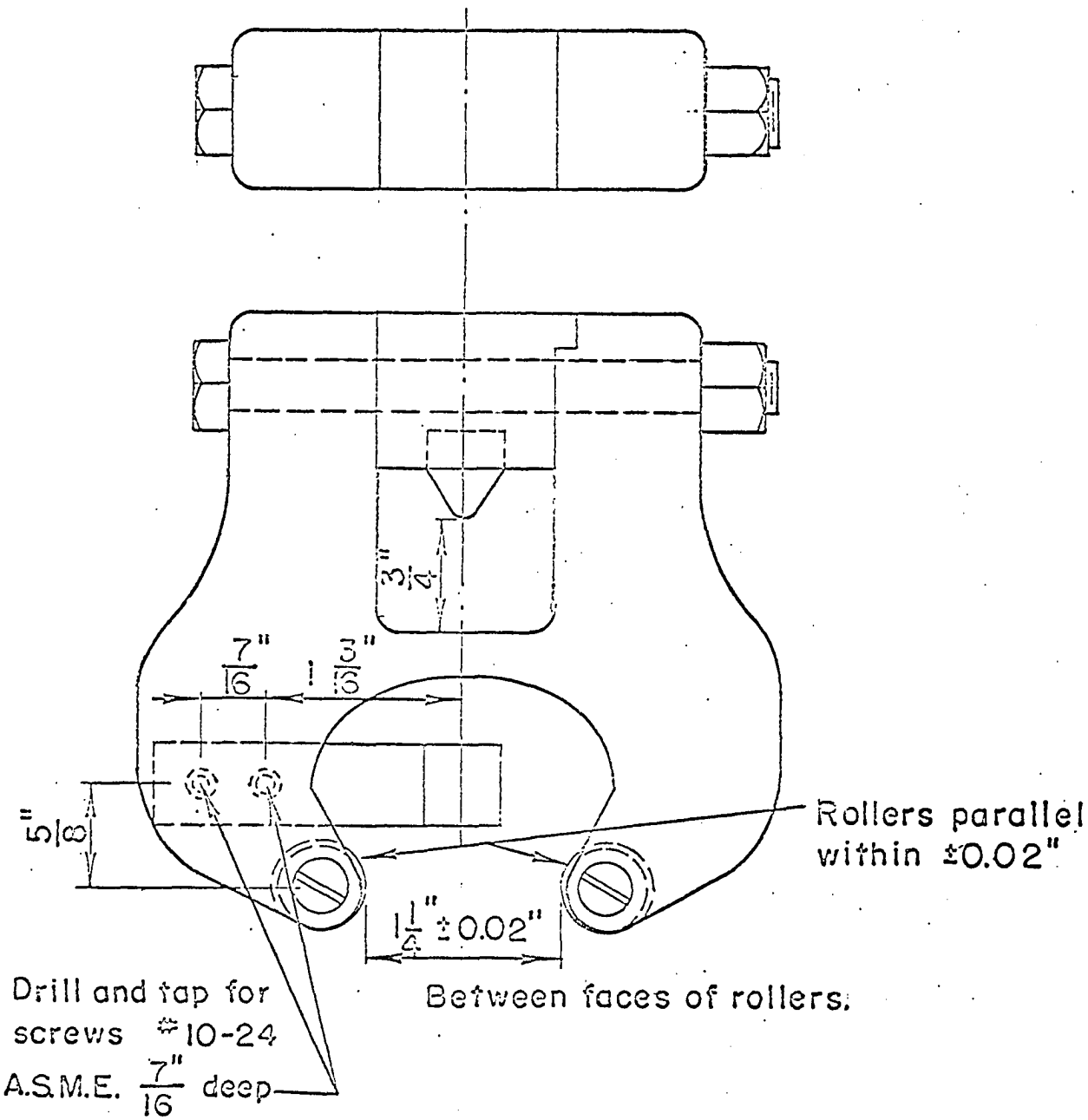

Fig. A1. Clips for briquet testing machine

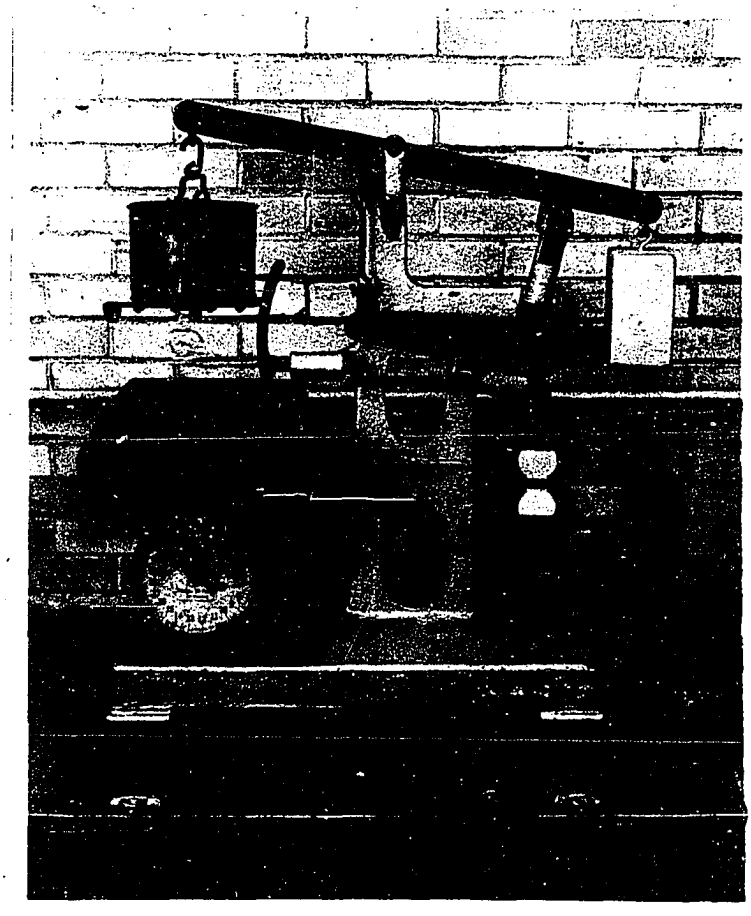

Figure A2. Speciman at right in tensile strength testing machine. Scales show a figure equivalent to over 1000 p.s.i. as strength of the glued joint

APPENDIX C. LYOPHILIZATION METHOD

The term lyophilization is more commonly used by a biochemist. By lyophilization is meant freeze drying -- the term that is more often used by a chemical engineer. This was conveniently carried out by firstly freezing the 10 to 40% (wet) suspension of the sample in water. Two 1000 ml flasks containing two such samples were connected (standard joint) to a specially designed long-necked 3000 ml capacity flask (14" long), which had an opening for connecting to the vacuum pump. The empty flask was immersed up to 10" of its length from bottom in a mixture of dry ice and acetone, taken in a Dewar flask. At the low temperature of -70^{o}, the average mean free path of water molecules becomes much extended, thus facilitating the direct ice to vapor conversion. Also the evaporation process is so rapid that the flasks containing samples remain at the freezing point. The vacuum pump should be able to evacuate to less than 0.1mm of mercury. Time required is usually 8 hours but overnight period is the more frequent one. In general, the products obtained after lyophilization are fluffy in nature.